Instructions to Peer Reviewers for Reviewing IRIS Summaries and Supporting Documentation

The U.S. EPA is conducting a peer review of the scientific basis supporting the health hazard and dose response assessments for the subject chemical that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). Materials to be reviewed include the summary information that will appear on IRIS (the inhalation reference concentration [RfC], oral reference dose [RfD], and cancer assessment) and the supporting document, the Toxicological Review, which will also be made available to the public.

A listing of Agency Guidelines and Methodologies that were used in the development of these hazard and dose-response assessments included the following: Guidelines for Carcinogen Risk Assessment (1986), Proposed Guidelines for Carcinogen Risk Assessment (1996), Guidelines for Developmental Toxicity Risk Assessment, Proposed Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity, Proposed Guidelines for Neurotoxicity Risk Assessment, Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry, Recommendations for and Documentation of Biological Values for Use in Risk Assessment and Use of the Benchmark Dose Approach in Health Risk Assessment. Copies of these documents (and/or their relevant sections) will be made to the reviewer upon request.

Peer review is meant to ensure that science is used credibly and appropriately in derivation of these dose-response assessments. You have been chosen as an expert on the chemical under consideration, on a scientific discipline related to at least one of the assessments, or in the field of risk assessment. At least three peer reviewers per chemical are being chosen to review the scientific basis of these draft dose-response assessments before they are forwarded on to EPA's Consensus Review for final approval and adoption by the EPA. These hazard and dose-response assessments will then appear on IRIS and become available as Agency consensus health effect information.

The primary function of the peer reviewer should be to judge whether the choice, use, and interpretation of data employed in the derivation of the assessments is appropriate and scientifically sound. This review is not of the recommended Agency risk assessment guidelines or methodologies used to derive cancer or RfD/C assessments as these have been reviewed by external scientific peers, the public, and EPA Science Advisory Boards. The reviewer's comments on the application of these guidelines/methodologies within the individual assessments is, however, welcomed and encouraged. For example, the reviewer may ascertain whether or not there is data sufficient to support use of other than default assumptions for areas such as sensitive subpopulations or linear cancer extrapolation. The reviewer may also have opinions on other areas of uncertainty such as subchronic to chronic duration (when only a subchronic study is available) or an incomplete data base but should focus on the specific area of uncertainty rather than on the magnitude of the overall estimate.

Below are two groups of questions regarding this review. The first is a set of general

questions that are meant to guide you through your review. It is not imperative that you specifically answer each question of this group. The second group of questions, however, are specific to hexachlorocyclopentadiene (HCCPD) and deal with areas of scientific controversy or uncertainty in which the Agency may have to make a scientific judgment. Your input to this set of questions is considered vital to the review process.

Questions for IRIS Peer Reviewers - General

1. Are you aware of any other data/studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of the adverse health effects, both cancer and noncancer, of this chemical?

2. For the RfD and RfC, has the most appropriate critical effect been chosen (i.e., that adverse effect appearing first in a dose-response continuum)? For the cancer assessment, are the tumors observed biologically significant? relevant to human health? Points relevant to this determination include whether or not the choice follows from the dose-response assessment, whether the effect is considered adverse, and if the effect (including tumors observed in the cancer assessment) and the species in which it is observed is a valid model for humans.

3. Have the noncancer and cancer assessments been based on the most appropriate studies? These studies should present the critical effect/cancer (tumors or appropriate precursor) in the clearest dose-response relationship. If not, what other study (or studies) should be chosen and why?

4. In the IRIS Summary document, studies included in the RfD and RfC under the heading "Supporting/Additional studies" are meant to lend scientific justification for the designation of critical effect by including any relevant pathogenesis in humans, any applicable mechanistic information, any evidence corroborative of the critical effect, or to establish the comprehensiveness of the data base with respect to various endpoints (such as reproductive/developmental toxicity studies). Should other studies be included under the "Supporting/Additional" category? Should some studies be removed?

5. For the noncancer assessments, are there other data that should be considered in developing the uncertainty factors or the modifying factor? Do the data support the use of different values than those proposed?

6. Do the confidence statements and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects (cancer and noncancer) to humans, and the comprehensiveness of the data base? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

Questions for IRIS Peer Reviewers - Specific for Hexachlorocyclopentadiene (HCCPD)

1. Regarding the RfD derivation

a) The RfD was based on the critical effect of forestomach lesions, as a manifestation of chronic irritation, in rats from a chronic gavage study by Abdo et al. (1984). Has sufficient justification been provided for the critical effect? Please elaborate on your response. If you disagree with the critical effect, please provide support for a different critical effect.

b) Does the Toxicological Review provide sufficient justification for a subchronic to chronic uncertainty factor of 3 (rather than the default value of 10) for the derivation of the RfD? Please explain. If you disagree with the uncertainty factor of 3, please provide the rationale and justification for a different uncertainty factor.

2. Regarding the RfC derivation

a) The RfC was based on the critical effect of suppurative inflammation of the nose in mice from a chronic inhalation study by NTP (1994). Has sufficient justification been provided for the principal study and critical effect? Please elaborate on your response, and, if you disagree, provide rationale for other choices.

3. Regarding the cancer classification

a) Has sufficient justification been provided for the carcinogen group classification for HCCPD? If you disagree with the classification, please provide the rationale and evidence for a different classification.

RECOMMENDATIONS

Based on your reading and analysis of the information provided, please identify your overall recommendation for the IRIS materials you have reviewed as

- acceptable as is
- acceptable with minor revision (as indicated)
- acceptable with major revision (as outlined)
- not acceptable

IRIS SUMMARY

0059
Hexachlorocyclopentadiene (HCCPD); CASRN 77-47-4; 00/00/00

Health assessment information on a chemical substance is included in IRIS only after a comprehensive review of chronic toxicity data by U.S. EPA health scientists from several Program Offices, Regional Offices, and the Office of Research and Development. The summaries presented in Sections I and II represent a consensus reached in the review process. Background information and explanations of the methods used to derive the values given in IRIS are provided in the Background Documents.

STATUS OF DATA FOR Hexachlorocyclopentadiene (HCCPD)

File First On-Line 00/00/00

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	on-line	00/00/00
Inhalation RfC Assessment (I.B.)	on-line	00/00/00
Carcinogenicity Assessment (II.)	on-line	00/00/00

_I. CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

_I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name -- Hexachlorocyclopentadiene (HCCPD)
CASRN -- 77-47-4
Last Revised -- 00/00/00

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an

appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

_1.A.1. ORAL RfD SUMMARY

The current RfD for HCCPD is a reevaluation of an assessment placed on-line on 09/01/1990. Although the current assessment used benchmark dose modeling for the dose-response analysis, the resulting RfD is similar to that reported in the 1990 assessment.

Critical Effect	Benchmark Doses	UF	MF	RfD
Chronic irritation in Rat Subchronic Gavage Bioassay (Abdo et al., 1984)	$BMDL_{10}$: 6 mg/kg/day BMD_{10}: 11 mg/kg/day	1000	1	6E-3 mg/kg/day

$BMDL_{10}$ - 95% Lower confidence limit on the maximum likelihood estimate of the dose corresponding to 10% risk.
BMD_{10} - Maximum likelihood estimate of the dose corresponding to 10% risk.

_I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

There are no chronic oral human studies or animal studies available for dose-response assessment. The only available subchronic oral bioassay was that by Abdo et al. (1984). The study was well conducted, but lacking in the design since hematological, clinical chemistry and urinalysis parameters were not investigated as required by current test guidelines (U.S. EPA, 1998). The study examined six dose levels in two species and obtained corroborative results.

Abdo, KM; Montgomery, CA; Kluwe, WM; et al (1984). Toxicity of hexachlorocyclopentadiene: subchronic (13-week) administration by gavage to F344 rats and B6C3F1 mice. J. Appl. Toxicol. 4: 75-81.

F344 rats (10/sex/dose) were administered 0, 10, 19, 38, 75, or 150 mg HCCPD/kg bw via corn oil gavage 5 days/week for 13 weeks. B6C3F1 mice were treated on the same regimen, but at doses of 0, 19, 38, 75, 150, or 300 mg/kg. Stability of the gavage mixture, or the frequency of preparation, was not reported. Standard bioassay data including body weights, organ weights, pathology, and histopathology were collected.

Mortality attributed to HCCPD occurred in six male rats in the 150 mg/kg group and in one male rat in the 75 mg/kg group. Other deaths were associated with gavage error, but the authors suggested that HCCPD may have been a contributor. A dose-related increase in the incidence of forestomach lesions started occurring in female rats at 19 mg/kg and in males at 38 mg/kg. Lesions were characterized by hyperplasia, acanthosis, and hyperkeratosis of the epithelial surface of the forestomach and increased mitotic activity in the basal layer of the epithelium. The forestomach lesions ranged from minimal to marked in severity and were focal to diffuse in distribution. Toxic nephrosis was noted in both sexes at 38 mg/kg and higher. Kidney lesions were predominantly limited to the terminal portion of the proximal convoluted tubules in the inner cortex and were characterized by dilated tubules and epithelial changes consisting of cytomegaly, karyomegaly, and anisokaryosis with nuclear and cytoplasmic vacuolization. Decreased body weights were noted in males at 38 mg/kg and in females at 75 mg/kg.

Mortality was observed in mice at 300 mg/kg, and was greater for males (10/10) than for females (3/10). Forestomach lesions were found in both sexes at 38 mg/kg. Lesions progressed to black foci, red cysts and ulceration at 150 mg/kg. Toxic nephrosis, which was observed beginning at 75 mg/kg, occurred only in the female mice.

The forestomach lesions, which are indicative of irritation, were chosen as the critical effect since the lesions occurred at lower doses than the toxic nephrosis and because the toxic nephrosis did not appear to be dose-related. The irritant effects on the forestomach are consistent with the observation of dermal irritation (HEW, 1978) and other portal of entry effects from HCCPD exposure (NTP, 1994). Female rats were more susceptible to the forestomach irritation than male rats or either sex of mice. The incidence of forestomach histopathology in female rats was 0/10, 0/10, 2/10, 5/10, 9/10, and 9/10 for the 0, 10, 19, 38, 75, or 150 mg/kg doses, respectively.

_I.A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF -- Chronic studies are preferred for RfD development. To account for the uncertainty in using a subchronic study for RfD derivation, a UF of 3 is applied. This UF was derived from the ratio of subchronic to chronic NOAELs for the mouse inhalation studies (NTP, 1994). This approach is justified by the fact that HCCPD produces local effects by both routes of exposure. The subchronic NOAEL to chronic NOAEL ratio from NTP (1994) was 0.8 for respiratory effects in rats while the ratio for mice was 3. To be conservative, 3 was chosen as the

subchronic to chronic UF for the RfD. Since there are no data available on which to base a pharmacokinetic or pharmacodynamic comparison of rodents to humans, the default UF of 10 is used for interspecies extrapolation. There are no data documenting the nature and extent of variability in human susceptibilities to HCCPD, so the default UF of 10 is used to protect sensitive human subpopulations. The database for HCCPD includes studies of genotoxicity, developmental toxicity, systemic toxicity, and cancer, but no two-generation reproductive studies are available. An additional UF of 3 is added for this database deficiency. Thus, the total UF is 1000.

MF -- None

_I.A.4. ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

The forestomach lesions observed in the critical study are consistent with the general portal of entry effects of this compound. Respiratory tract damage (NTP, 1994) and skin lesions (HEW, 1978) are observed during inhalation and dermal exposures, respectively. The kidney is a target organ in oral studies (Abdo et al.,1984) and in one (Clark et al., 1982) of three inhalation studies which noted mild degenerative kidney and liver lesions in rats at doses which also produce respiratory tract necrosis. One report of accidental human exposure suggests that the liver may also be a target organ (Kominsky et al., 1980). HCCPD was not teratogenic by oral gavage in rats, mice, or rabbits (Chernoff and Kavlock, 1983; Murray et al., 1980; Goldenthal et al., 1978).

_I.A.5. CONFIDENCE IN THE ORAL RfD

Study -- Medium
Data Base -- Low
RfD -- Low

The confidence in the principal study is medium. Although it was well conducted, an adequate number of doses were examined, and corroborative results in two species were obtained, the design was lacking because no data on hematology, clinical chemistry or urine analyses were collected. In addition, there are no supporting subchronic or chronic oral studies with which to compare the effects noted. Teratogenic studies are available for three species, but confidence in the database in low due to the lack of a two-generation reproductive study. Thus, confidence in the RfD can also be considered low.

_I.A.6. EPA DOCUMENTATION AND REVIEW OF ORAL RfD

Source Document--U.S. EPA, 2000.

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to the Toxicological Review for

Hexachlorocyclopentadiene.

Agency Consensus Date --00/00/00

_I.A.7. EPA CONTACTS (ORAL RfD)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513)569-7254 (phone), (513)569-7159 (FAX), or RIH.IRIS@EPAMAIL.EPA.GOV (internet address).

_I.B. REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Hexachlorocyclopentadiene
CASRN -- 77-47-4
Last Revised -- 00/00/00

The inhalation Reference Concentration (RfC) is analogous to the oral RfD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). It is generally expressed in units of mg/m^3. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Inhalation RfCs were derived according to the Interim Methods for Development of Inhalation Reference Doses (EPA/600/8-88/066F August 1989) and subsequently, according to Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F October 1994). RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

_1.B.1. INHALATION RfC SUMMARY

The RfC is new to the IRIS file for HCCPD. The assessment placed on-line on 09/01/1990 did not include an RfC.

Critical Effect	Exposure Concentrations		UF	MF	RfC
Suppurative inflammation of the nose in Chronic Inhalation Study in B6C3F1 Mice (NTP, 1994)	NOAEL	0.56 mg/m^3	100	1	2E-4 mg/m^3
	$NOAEL_{ADJ}$[1]:	0.1 mg/m^3			
	$NOAEL_{HEC}$[2]:	0.024 mg/m^3			
	LOAEL:	2.23 mg/m^3			
	$LOAEL_{ADJ}$[1]:	0.4 mg/m^3			
	$LOAEL_{HEC}$[2]:	0.095 mg/m^3			

Conversion Factors and Assumptions --

[1]Conversion from intermittent exposure to continuous exposure: 0.56 mg/m³ × 6/24 hrs × 5/7 days = 0.1 mg/m³.

[2]Conversion to human equivalent concentration (HEC) for interspecies dosimetric adjustment. $NOAEL_{HEC}$ was calculated for an effect in the extrathoracic (ET) region. MV_A = 0.049 L/min, MV_H = 13.8 L/min, $(S_{ET})_A$ = 3 cm², $S(_{ET})_H$ = 200 cm². $RGDR_{ET} = (MV_A/(S_{ET})_A) / (MV_H/S(_{ET})_H) = 0.237$. $NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR_{ET}$ = 0.1 mg/m³ × 0.237 = 0.024 mg/m³

_I.B.2. PRINCIPAL AND SUPPORTING STUDIES (INHALATION RfC)

There are no chronic human inhalation studies suitable for dose-response assessment. The only chronic human studies available have used an insensitive endpoint such as mortality, and cannot differentiate the effects of HCCPD from those of other chemicals. Thus, the only chronic animal study available was chosen as the principal study. NTP (1994) reports well-conducted inhalation bioassays with two species and is suitable to evaluate dose-response.

NTP. (1994) Toxicology and carcinogenesis studies of hexachlorocyclopentadiene in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program Technical Report Series 437: 318.

Sixty rats or mice per sex were exposed to atmospheres containing 0, 0.11, 0.56, and 2.23 mg/m^3 HCCPD for 5 days/week for 2 years. Ten male and 10 female rats and mice from each exposure group were evaluated at 15 months. The stability of the compound was monitored throughout the study, and it was found that no degradation took place for up to 2 years. Standard bioassay data including body weights, organ weights, urinalysis and histopathology were collected.

Exposure to HCCPD did not significantly affect survival of rats or mice, but the decrease in survival of female mice approached statistical significance in the 2.23 mg/m^3 group due to suppurative inflammation of the ovary. Body weights of rats were unchanged by HCCPD exposure, but body weights of male and female mice were reduced in the 2.23 mg/m^3 group.

Exposure was associated with a yellow-brown granular pigmentation within the cytoplasm of epithelial cells lining the respiratory tract in both rats and mice. In female rats, significant increases in the incidence of squamous metaplasia of the larynx were seen in the 0.11 and 2.23 mg/m^3 groups. The lesion, described as stratified squamous epithelium several cell layers thick in areas usually lined by columnar epithelium, was considered to be of minimal severity in all groups. Because there is individual variation in the location of the transition between squamous and columnar epithelia and in obtaining consistent tissue sections in the treated rats, NTP indicated that the significance of this metaplasia is unknown. In addition, a dose-response relationship was not evident. Thus, the NOAEL for rats was 2.23 mg/m^3 HCCPD and there was no LOAEL.

Female mice exhibited a dose-related increase in the incidence of suppurative ovarian inflammation that was significantly different from controls at 0.56 and 2.23 mg/m^3 HCCPD. The lesion was similar to other utero-ovarian infections observed in mice in NTP studies and is apparently caused by Klebsiella infection. At 2.23 mg/m^3 HCCPD, increases in suppurative inflammation of the nose were noted in both male and female mice during the interim evaluation at 15 months and at study termination. Suppurative inflammation of the nose in mice was chosen as the critical effect since it was the only respiratory tract effect that occurred in either species. Neither sex of mice was clearly more sensitive to the effect than the other, so both sexes were used for the dose-response analysis. The incidence of this effect was 4/99, 0/100, 4/100, and 76/98 in the 0, 0.11, 0.56, and 2.23 mg/m^3 groups, respectively. Thus, the NOAEL in mice for suppurative inflammation of the nose was 0.56 mg/m^3 HCCPD and the LOAEL was 2.23 mg/m^3.

A 13 week study (NTP, 1994) provides supporting evidence that the respiratory tract is the major target of inhalation exposure to HCCPD. That study exposed the same strains of animals (10 per sex per species) for 5 days per week, 6 hours per day, to atmospheres containing 0, 0.45, 1.7, 4.5, 11, or 22 mg/m^3 HCCPD. No chemical-related differences in hematology, clinical chemistry, or urinalysis parameters were reported in exposed rats.

All rats in the 11 and 22 mg/m^3 groups died. Necropsy of rats in the 11 and 22 mg/m^3 groups revealed extensive coagulation necrosis in the respiratory epithelium of the nose, larynx, trachea, bronchi and bronchioles. Necrosis was accompanied by inflammatory signs such as vascular congestion, edema, fibrin accumulation, and neutrophil and mononuclear cell infiltration. Male rats in the 4.5 mg/m^3 group exhibited significantly increased absolute and relative lung weights, as well as necrotizing and suppurative inflammation of the nose, bronchus, and bronchioles and squamous metaplasia of the nose. The squamous metaplasia was focal in nature, generally observed on the tips of the turbinates, and characterized by stratification of the epithelium to form three to four poorly defined layers of flattened, nonkeratinized polygonal cells. Female rats seemed to be less sensitive. At the 4.5 mg/m^3 exposure, the only nasal effects were suppurative inflammation and fewer females than males exhibited necrotizing and suppurative inflammation of the bronchus and bronchioles. Since no respiratory lesions were seen at exposures lower than 4.5 mg/m^3 HCCPD, the NOAEL was 1.7 mg/m^3. This is similar to the NOAEL of 2.3 mg/m^3 observed for rats in the chronic study.

All mice in the 11 and 22 mg/m^3 groups died within five weeks. Before the end of the study, seven deaths occurred in the 4.5 mg/m^3 group, one death occurred in the 1.7 mg/m^3 group, and three deaths occurred in the 0.45 mg/m^3 group. Six deaths in the female control group were attributed to a defective feeder. No chemical-related differences in hematology, clinical chemistry, or urinalysis parameters were reported in exposed mice. Males in the 0.45 mg/m^3 group exhibited a statistically significant decrease in weight which was not toxicologically significant (i.e., <10%). Body weights of exposed animals were similar to controls in all other groups.

As evidence by a somewhat lower frequency of effects, mice were not as sensitive to the respiratory toxicity of HCCPD as rats. Male mice exhibited significant increases in suppurative inflammation of the nose and squamous metaplasia of the trachea at 4.5 and 11 mg/m^3, and acute necrosis and suppurative inflammation of the nose, acute necrosis of the larynx, trachea, and lung, and congestion of the lung at 22 mg/m^3. Female mice had serous inflammation of the nose at 4.5 mg/m^3, and suppurative inflammation of the nose, squamous metaplasia of the larynx and trachea, and necrotizing inflammation of the lung at 11 mg/m^3. At the highest dose, female mice presented the same spectrum of effects as male mice. No respiratory effects were observed in mice at 1.7 mg/m^3. The chronic study observed a NOAEL of 0.56 mg/m^3 HCCPD in mice. The 1.7 mg/m^3 NOAEL in the 13 week study (NTP, 1994) supports the use of the chronic study (NTP, 1994) as the principal study since a lower NOAEL was observed.

Clark, DG; Pilcher, A; Blair, D; et al. (1982) Thirty week chronic inhalation study of hexachlorocyclopentadiene (HEX) in rats. Group Research Report SBGR.82.051. NTIS/OTIS43022.

This study also showed that the respiratory tract is the major target organ of inhaled HCCPD. Wistar rats inhaled, via whole body exposure, 0, 0.05, 0.1 or 0.5 ppm (conversion of 1 ppm = 11.3 mg/m^3 yields 0, 0.56, 1.1, or 5.6 mg/m^3, respectively) HCCPD for 6 hours/day, 5 days/week, for 30 weeks and recovered from exposure for 14 weeks. Chemical purity of the compound decreased from 96% to 90% during the course of the study due to oxidation. Bronchopneumonia was noted in four males and two females which died during exposure to 5.6 mg/m^3. Two of the deceased rats had enlarged adrenals and the thorax contained watery or bloodstained fluid.

Males in the 1.1 mg/m^3 and 5.6 mg/m^3 groups had significantly higher mean erythrocyte counts, hemoglobin concentrations, hematocrit and absolute numbers of neutrophils, and significantly lower lymphocyte counts than the controls. Mean absolute numbers of lymphocytes were lower in females at the 5.6 mg/m^3 dose.

Body weights of males from the 5.6 mg/m^3 dose group were significantly lower than controls. Several increases in body weights in females exposed to HCCPD compared to controls were noted, but by the end of the recovery period, body weights of females exposed to 1.1 and 5.6 mg/m^3 HCCPD were significantly lower than controls. Kidney weights were significantly increased in females in the 5.6 mg/m^3 group after exposure for 30 weeks. Male heart weights

were decreased at 30 weeks in the 5.6 mg/m^3 group. The organ weight effects were not considered to be biologically significant by the study authors.

Rats at the 5.6 mg/m^3 dose showed pulmonary degenerative changes including epithelial hyperplasia, edema, and sloughing of the bronchiolar epithelium in both sexes and epithelial ulceration and necrosis in the males. No degenerative changes in the lungs were observed in the 0.56 or 1.1 mg/m^3 dose groups. The 1.1 mg/m^3 NOAEL for respiratory effects in rats in this study supports the use of NTP (1994) as the principal study since a lower NOAEL was observed in mice. Clark et al. (1982) also observed mild degenerative changes in the liver and kidney of rats in the 5.6 mg/m^3 group. The authors suggested that the toxic action of HCCPD involves an extreme local irritation of the respiratory tract that causes death by respiratory failure following bronchopneumonia. Clark et al. (1982) considered the mild degenerative changes in the livers and kidneys of a few animals unlikely to contribute significantly to HCCPD's toxicity in the rat.

Rand, GM; Nees, PO; Calo, CJ; et al. (1982) Effects of inhalation exposure to hexachlorocyclopentadiene on rats and monkeys. J Toxicol Environ Health 9:743-760.

Sprague-Dawley rats and cynomolgus monkeys inhaled, via whole-body exposure, 97.7% HCCPD at 0, 0.01, 0.05 or 0.20 ppm (conversion of 1 ppm = 11.3 mg/m^3 yields 0, 0.11, 0.56, or 2.2 mg/m^3, respectively) for 6 hours/day, 5 days/week, for 14 weeks. Each exposure group contained 40 male and 40 female rats, and six male and six female monkeys.

Rand et al. (1982) reported no mortalities or adverse clinical signs in monkeys at any exposure level. Body weight gain and food consumption were not significantly different between groups. Pulmonary function tests (blood gas analysis, lung mechanics, lung ventilation) were normal. No eye lesions were noted, and no exposure-related changes were noted in hematology, clinical chemistry, urinalysis, organ weights, macroscopic pathology, and histopathology. Since no adverse effects were noted, the NOAEL for monkeys was 2.2 mg/m^3 HCCPD.

The only significant clinical sign reported in male rats was dark, red eyes observed in the 0.56 and 2.2 mg/m^3 dose groups. Ophthalmoscopic examination revealed no eye lesions. There were no exposure-related changes in body weight gain, food or water consumption, or urinalysis. After 12 weeks of exposure, there were slight, occasionally statistically significant increases in hemoglobin, red blood cell count, and mean corpuscular hemoglobin concentration with a corresponding reduction in the mean cell volume in males at 0.11 and 2.2 mg/m^3 and in females at 0.56 and 2.2 mg/m^3. The authors observed similar effects in a range-finding study and considered them to be indicative of impaired respiratory function. There were no other effects on hematology. Statistically significant decreases in mean liver weight occurred in all treatment groups and in kidneys of all treated males after 13 weeks of exposure. No treatment related gross pathology or histopathology was observed. Since the changes in hematologic parameters were not dose-related, and the kidney and liver weight changes were not accompanied by pathology, the NOAEL for rats was 2.2 mg/m^3. This study supports the use of NTP (1994) as the principal study since a lower NOAEL was observed in mice.

_I.B.3. UNCERTAINTY AND MODIFYING FACTORS (INHALATION RfC)

UF -- The default uncertainty factor for interspecies extrapolation is 10. Half of that factor, $10^{1/2}$, or 3, reflects the pharmacokinetic component of interspecies uncertainty and half represents the pharmacodynamic component of interspecies uncertainty. The pharmacokinetic component of interspecies uncertainty is accounted for by the dosimetric adjustment which converts animal exposure concentrations of HCCPD to human equivalent concentrations (HEC). Thus, an uncertainty factor of 3 is employed for interspecies extrapolation to reflect the pharmacodynamic component of interspecies uncertainty. There are no data documenting the nature and extent of variability in human susceptibilities to HCCPD, so the default UF of 10 is used to protect sensitive human subpopulations. A factor of 3 is applied for an incomplete database since the inhalation database lacks developmental and two-generation reproductive studies. The total UF is 100.

MF -- None

_I.B.4. ADDITIONAL STUDIES/COMMENTS (INHALATION RfC)

Portal of entry irritation effects due to HCCPD are also observed for oral (Abdo et al, 1984) and dermal (HEW, 1978) routes of exposure. Severe lung irritation and hemorrhaging were observed in rats acutely exposed to high concentrations of HCCPD (Wazeter and Geil, 1972). Tracheobronchial irritation was reported in humans after accidental exposure to high levels of HCCPD vapor (Kominsky et al., 1980).

_I.B.5. CONFIDENCE IN THE INHALATION RfC

Study -- High
Data Base -- Medium
RfC -- Medium

The overall confidence in the RfC assessment is medium. The confidence in the principal study is high because it was well-designed, well-conducted, and followed standard guidelines for toxicity studies of chronic duration. The overall confidence in the database is medium. Although there are two subchronic studies which verify that the respiratory tract is the major target organ, the database lacks reproductive/developmental studies in rodents following inhalation exposure to HCCPD. Oral teratogenicity studies in three species, however, indicate that HCCPD is not teratogenic at doses (i.e., 75 mg/kg) higher than those which cause portal of entry irritation (i.e., 19 mg/kg). This suggests that the possible teratogenic effects of inhaled HCCPD may be less sensitive than respiratory tract effects.

_I.B.6. EPA DOCUMENTATION AND REVIEW OF INHALATION RfC

Source Document--U.S. EPA, 2000.

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to the Toxicological Review for Hexachlorocyclopentadiene.

Agency Consensus Date –00/00/00

_I.B.7. EPA CONTACTS (INHALATION RfC)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513)569-7254 (phone), (513)569-7159 (FAX), or RIH.IRIS@EPAMAIL.EPA.GOV (internet address).

_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name -- Hexachlorocyclopentadiene (HCCPD)
CASRN -- 77-47-4
Last Revised -- 00/00/00

Section II provides information on three aspects of the carcinogenic assessment for the substance in question; the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of applying a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. The third form in which risk is presented is a concentration of the chemical in drinking water or air associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in The Risk Assessment Guidelines of 1986 (EPA/600/887/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

_II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

The current carcinogenicity assessment for HCCPD is a revision of the assessment placed in IRIS on 09/01/1990.

_II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

Human occupational studies indicate that exposure to HCCPD does not result in an

increase in deaths from cancer (Brown et al., 1980; Buncher et al., 1980; Shindell, 1980, 1981; Wang and MacMahon, 1979), but these studies are limited by the exposure of cohorts to other chemicals, short follow-up periods, small number of person-years, and lack of data on cigarette smoking. One 2-year inhalation carcinogenesis study in animals reported no exposure-related increase in the incidence of tumors in rats or mice at doses up to 2.2 mg/m^3 (NTP, 1994). NTP (1994) concluded that there was no evidence of carcinogenic activity of HCCPD. No oral cancer studies have been performed.

A number of in vitro and in vivo mutagenicity assays with HCCPD have been negative (EPA, 1984; NTP, 1994; Litton, 1978; Brat, 1983; Zimmering et al., 1985; Mason et al., 1992) The only positive result for mutagenicity was a significant increase in sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells (NTP, 1994).

The apparent inability of HCCPD to cause genotoxic effects, and the lack of evidence for both human and animal carcinogenicity, justify the conclusion that HCCPD is not likely to present a human cancer risk. According to the existing Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), evaluation of the weight-of-evidence for carcinogenicity to humans indicates that HCCPD is most appropriately categorized as Group E, Evidence of Noncarcinogenicity to Humans. This characterization is based on no evidence of cancer in limited human studies and no evidence of cancer in well-conducted animal studies. In accordance with U.S. EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996), HCCPD is not likely to be a human carcinogen. This characterization is based on the lack of increased deaths from cancer in limited human studies, no evidence of cancer in rodents, and lack of mutagenicity.

_II.A.2. HUMAN CARCINOGENICITY DATA

Inadequate. Several retrospective mortality studies have been conducted on employees in plants that either produced HCCPD or used it in the manufacture of chlorinated pesticides. These studies, however, are inadequate to assess carcinogenicity of hexachlorocyclopentadiene alone because they do not estimate exposure levels to the chemical or correlate excess deaths with exposure. The studies are also limited by exposure of cohorts to other chemicals, short follow-up periods, small number of person-years, and lack of data on cigarette smoking.

Shindell and Associates (1980) conducted a mortality study of 783 workers employed at least 3 months between January 1, 1946 and December 31, 1979 at the Velsicol Chemical Corporation plant in Marshall, IL. This plant manufactured synthetic chlorinated hydrocarbon insecticides using HCCPD as an intermediate. The vital status of 97.4% of the cohort was known. The causes of death examined included malignant neoplasms, diseases of the heart and circulatory system, cerebrovascular disease, trauma, and others. The number of observed deaths in each category was compared to the number of expected deaths calculated from race- and sex-specific U.S. mortality rates for appropriate 5-year periods. No excess deaths related to any specific job class or product were seen. Except for "other deaths" in females, the number of

deaths observed appear lower than the number expected. The 22 deaths from cancer included brain, kidney, liver, lung, and digestive system cancers; 8 of the 22 cancer deaths were from lung cancer. The number of expected deaths for each specific cancer was not calculated.

Shindell and Associates (1981) conducted a mortality study with 1115 workers employed for at least 3 months between January 1, 1952 and December 31, 1979 at the Velsicol Chemical Corporation plant in Memphis, TN. This plant manufactured synthetic chlorinated hydrocarbon insecticides using HCCPD as an intermediate. The vital status of 92.8% of the cohort was known. The study design was the same as that of the Shindell and Associates (1980) study described above. Deaths from strokes and from trauma showed an increase over the number of expected deaths, but the increases were not statistically significant. The distribution of the standard mortality ratio of cancer deaths by cancer site and job showed a nonsignificant excess of lung cancer in maintenance workers. The study authors concluded that there was no pattern of neoplasia suggestive of job-related risk.

Wang and MacMahon (1979) also conducted a retrospective mortality study of the workers at the Velsicol Chemical Corporation plants in Marshall, IL and Memphis, TN. The study group included 1403 males who worked at either plant longer than 3 months before the spring of 1976. Person-years were calculated for January 1, 1946 to June 30, 1976 for Marshall employees and for January 1, 1952 to December 31, 1976 for Memphis employees. Approximately 34% of the subjects had fewer than 10 years follow-up and 36% had 20 or more years of follow-up study. Expected deaths for these person-years were calculated from white male national mortality rates through 1975. Observed deaths due to all causes were significantly fewer than expected. Deaths due to cerebrovascular disease, however, were statistically elevated over those expected. Deaths due to all cancers were fewer than expected, but deaths due to lung cancer were greater than expected, although not significantly. There was no relationship between lung cancer deaths and duration of exposure to HCCPD or duration of follow-up. No data on cigarette smoking are available for this study group. There was one death each from cancer of the liver, bladder, prostate, and central nervous system.

A mortality study was performed involving cohorts which overlapped the one used in the Wang and MacMahon study (1979) but extended the follow-up period (Brown et al. 1980). Different cohorts from four chemical plants that manufactured organochlorine pesticides were used in this study. These cohorts comprised all workers at each plant who had worked at least 6 months prior to December 31, 1964. Causes of deaths among the cohorts occurring prior to December 31, 1976 were recorded. Observed deaths in the cohorts were far fewer than expected, reflecting the healthy worker effect. The expected value was calculated using U.S. white-male cause-specific mortality rates, but the report did not specify the ethnicity or sex of the employees studied. The increase in cerebrovascular disease, observed in the Wang and MacMahon study (1979) was not reported in this study. A deficit in deaths from all malignant neoplasms in each plant was observed, but the numbers of workers dying from cancer were too few to provide statistically significant values. There were slight, but not statistically significant, increases in stomach cancer deaths in one plant, and slight excesses of cancer of the esophagus, cancer of the rectum, liver cancer, and cancer of the lymphatic and hematopoietic system in

another plant. However, exposure to multiple organochlorine compounds in each of the plants precludes linking these cancer cases with exposure to HCCPD or any other individual compound.

Buncher et al. (1980) conducted an occupational mortality study with 341 workers at the Hooker Chemical Corporation plant in Montague, MI. The plant produced HCCPD and other chlorinated hydrocarbons. Employees who had worked at least 90 days between October 1, 1953 and December 31, 1974 were included in the cohort. Follow-up was through December 31, 1978. Expected deaths were calculated using sex-, age- and year-specific U.S. mortality rates. Deaths due to all causes, all cancers, diseases of the circulatory system, diseases of the digestive system, and external causes were all fewer than expected. The six observed cancer deaths included one of the kidney, and two of the respiratory system. The ratio of observed-to-expected deaths for the respiratory cancers (0.87) and colon cancer (1.75) are near 1.0 and are not statistically significant. The remaining cancers have ratios greater than or equal to 5; however, the small numbers of deaths prevents drawing a firm conclusion. The short follow-up period is also a limitation in this study.

_II.A.3. ANIMAL CARCINOGENICITY DATA

No evidence of carcinogenicity. NTP (1994) conducted a 2-year inhalation study with rats and mice, and concluded that HCCPD exhibited no evidence of carcinogenic activity (NTP, 1994). Groups of 60 animals per sex per species were exposed, via whole body inhalation, for 5 days per week, 6 hours per day, to 0, 0.11, 0.56, or 2.23 mg/m^3 HCCPD. The study was well designed and involved two rodent species and an appropriate number of subjects at each dose. No exposure-related increases in neoplasms were seen in male or female rats or mice. In male rats, a significant increase in the incidence of pars distalis adenoma of the pituitary (33/50, or 66%) was seen in the 2.3 mg/m^3 group. Since the historical control incidence of pars distalis adenoma in male F344/N rats from other NTP inhalation studies is 60%, with a range of 45–68%, this effect was not considered to be related to HCCPD exposure.

_II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

The weight-of-evidence for mutagenicity indicates that HCCPD is not mutagenic. A battery of genotoxicity studies performed by the NTP yielded generally negative results for HCCPD (NTP, 1994). NTP (1994) confirmed previous negative results for HCCPD in the Ames test (Shell Oil Company, 1983; Industrial Biotest Laboratories, 1977). Negative results were also seen for changes in micronucleated erythrocyte frequency in B6C3F1 mice exposed to HCCPD for 13 weeks by inhalation, and for induction of sex-linked recessive lethal mutations in male *Drosophila melanogaster*. The negative results in *Drosophila melanogaster* confirmed those of other investigators (Mason et al.; 1992; Zimmering et al. 1985). HCCPD did not induce a significant increase in morphological transformation in BALB/3T3 cells and did not induce forward mutations in mouse lymphoma cells at non-cytotoxic concentrations (Litton Bionetics, Inc. 1978). Cytogenetic effects manifested as sister chromatid exchanges and chromosomal aberrations were observed in Chinese hamster ovary cells exposed to HCCPD with and without

S9 (NTP, 1994), but chromosome damage did not occur in metaphase stage rat liver (RL4) cells (Shell Oil Company 1983). HCCPD at subtoxic concentrations also did not induce DNA repair when incubated with rat hepatocytes *in vitro* (Brat, 1983).

_II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

Not available.

_II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

Not available.

_II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

_II.D.1. EPA DOCUMENTATION

Source Document --U.S. EPA, 2000.

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to the Toxicological Review for Hexachlorocyclopentadiene.

_II.D.2. EPA REVIEW (CARCINOGENICITY ASSESSMENT)

Not available.

_II.D.3. EPA CONTACTS (CARCINOGENICITY ASSESSMENT)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513)569-7254 (phone), (513)569-7159 (FAX), or RIH.IRIS@EPAMAIL.EPA GOV (internet address).

_III. [reserved]
_IV. [reserved]
_V. [reserved]

_VI. BIBLIOGRAPHY

Substance Name -- Hexachlorocyclopentadiene
CASRN --77-47-4
Last Revised -- 00/00/00

_VI.A. ORAL RfD REFERENCES

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_VII. REVISION HISTORY

Substance Name: Hexachlorocyclopentadiene
CASRN --77-47-4

Date	Section	Description
03/01/1989	I.A.6.	Verification date changed
09/01/1989	VI.	Bibliography on-line
11/01/1989	II.	Carcinogen assessment now under review
06/01/1990	IV.A.1.	Area code for EPA contact corrected
06/01/1990	IV.F.1.	EPA contact changed
09/01/1990	I.A.	Text edited
09/01/1990	II.	Carcinogen assessment on-line
09/01/1990	VI.C.	Carcinogen references added
08/01/1991	VI.A.	Citations clarified
08/01/1991	VI.C.	Citations clarified
01/01/1992	I.A.7.	Secondary contact changed
01/01/1992	IV.	Regulatory actions updated
04/01/1992	IV.A.1.	CAA regulatory action withdrawn
00/00/0000	I.A	Revised RfD
00/00/0000	I.B.	Added RfC
00/00/0000	II.	Revised carcinogen summary

_VIII. SYNONYMS

Substance Name -- Hexachlorocyclopentadiene
CASRN --77-47-4
Last Revised -- 00/00/00

77-47-4
C-56
GRAPHLOX
HCCP
HCCPD
HEX
HEXACHLORO-1,3-CYCLOPENTADIENE
HEXACHLOROPENTADIENE
PCL
PERCHLOROCYCLOPENTADIENE

TOXICOLOGICAL REVIEW

OF

HEXACHLOROCYCLOPENTADIENE

(CAS No. 77-47-4)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

May 2000

EXTERNAL REVIEW DRAFT

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. This document may undergo revisions in the future. The most up-to-date version will be available electronically via the IRIS Home Page at http://www.epa.gov/iris.

CONTENTS—TOXICOLOGICAL REVIEW FOR HEXACHLOROCYCLOPENTADIENE (CAS No. 77-47-4)

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard identification and dose-response assessment in IRIS pertaining to chronic exposure to hexachlorocyclopentadiene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of hexachlorocyclopentadiene.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose-response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

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Reviewers

This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC is analogous to the oral RfD. The inhalation RfC considers toxic effects for both respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3. The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgement of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ air breathed. Another form in which risks is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identifications and dose-response assessments for hexachlorocyclopentadiene has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for*

Developmental Toxicity Risk Assessment (U.S. EPA, 1991), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996), *Interim Policy for Particle Size and Limit Concentration Issues and Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995a), *Science Policy Council Handbook*: *Peer Review* (U.S. EPA, 1998a), and a memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategies employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

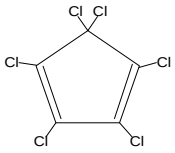
2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENT

Other names for hexachlorocyclopentadiene include C-56, hexachloro-1,3-cyclopentadiene, graphlox, HCCP, HCCPD, Hex, hexachloropentadiene, HRS 1655, NCI-C55607, PCL, and perchlorocyclopentadiene. It is predominately used as an intermediate in the production for many compounds used as dyes, resins, pharmaceuticals, flame retardants, insecticides, and polyester resins. Hexachlorocyclopentadiene (HCCPD) is also used to produce ketones, fluorocarbons, acids, esters, and shock-proof plastics.

HCCPD exists as a dense oily liquid, pale yellow to amber in color, at room temperature (melting point at -9°C, boiling point at 239°C). It has a pungent, unpleasant odor. Vapors are present at room temperature due to its high vapor pressure. HCCPD is soluble in organic nonpolar solvents, but only slightly soluble in water. HCCPD degrades in the presence of light

and may decompose to produce toxic fumes upon heating (HSDB, 1999). See Table 2-1 for selected chemical and physical properties of HCCPD

Table 2-1. Chemical and Physical Properties of Hexachlorocyclopentadiene

Properties	Values	Reference
Boiling point	239°C	HSDB, 1999
Melting point	-9°C	HSDB, 1999
Molecular weight	272.77	HSDB, 1999
Density	1.7019 at 25°C	HSDB, 1999
K_{oc}	4,265	U.S. EPA, 1999
Log K_{ow}	3.99	U.S. EPA, 1999
Solubility	2 mg/L water at 25°C	U.S. EPA, 1995b
Vapor pressure	0.08 mm Hg at 25°C	U.S. EPA, 1999
Henry's law coefficient	2.7×10^{-2} atm-cu m/mole	U.S. EPA, 1999
Chemical structure (C_5Cl_6)		

Conversion factor: 1 ppm = 11.3 mg/m^3; 1 mg/m^3 = 0.088 ppm (World Health Organization, 1991).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. Absorption

Yu and Atallah (1981) showed that HCCPD is poorly absorbed in rats following gavage administration. They administered single doses of 99% pure ^{14}C-HCCPD to 21 female and six male Sprague-Dawley rats (divided into groups of 1–3 per dose and time-point of analysis). Approximately 25 mg/kg ^{14}C-HCCPD dissolved in 0.5 ml corn oil was delivered via gavage

while 0.73 mg/kg HCCPD dissolved in 0.3 ml 20% Emulphor EL0620 saline solution was administered IV. Urine and fecal samples were obtained daily. Blood samples were obtained at various post-administration durations, with the first sample taken at 0.5 hours and the last sample taken at 24 hours.

The concentration of ^{14}C in blood following oral administration rose gradually to a maximum of 2.25±0.30 ppm HCCPD-equivalents at 4 hours post-dosing, and then fell to 0.95±0.16 ppm by 24 hours post-dosing. After IV administration, HCCPD-equivalents reached a maximum of 5.08±1.02 ppm at 0.5 hours post-exposure and dropped to 2.34±0.75 ppm at 24 hours. Despite the much lower dose given by IV, the area under the concentration-duration curve for the blood of IV-injected rats was 70 times that of gavaged rats. The body burden for IV-injected rats was ten times that of gavaged rats.

Lawrence and Dorough (1981) used one female Sprague-Dawley rat/dose to investigate the retention of 1.4, 17.3 and 37.4 µg ^{14}C-HCCPD /kg inhaled, via nose only exposure, over a one hour period. Retention of the compound was 84% and independent of dose. Lawrence and Dorough (1982) performed a similar experiment and reported 91% retention after 1.5 hours and 95% retention after 2 hours inhalation exposure to both low (1-5 µg/kg) and high (30-40 µg/kg) doses of ^{14}C-HCCPD. Lawrence and Dorough (1982) also measured the blood concentrations of ^{14}C after administration of 10 µg ^{14}C-HCCPD/kg via 0.5 ml corn oil gavage, nose only inhalation (one hour), and IV routes (in 0.2 ml dimethyl sulfoxide or 10:4:1 saline:propylene glycol:ethanol) and confirmed the results of Yu and Atallah (1981) which indicated poor absorption for the oral route. Peak ^{14}C blood concentrations for the oral route were approximately 1/5th that of the inhalation route and approximately 1/50th that of the IV route.

3.2 Distribution and Metabolism

Several studies were performed to determine distribution and metabolism of HCCPD after oral administration. Mehendale (1977) administered 5 µmole (~ 6 mg/kg in rats weighing 225–250 grams) of ^{14}C-HCCPD in 0.2 ml corn oil to male Sprague-Dawley rats via oral intubation. Urine and fecal samples were collected daily for seven days. The animals were then

sacrificed for collection of liver, kidneys, fat, lung, muscle, and blood tissues. After seven days, the kidneys retained 0.5% of the administered dose, the liver retained less than 0.5%, and the remaining tissues contained only trace amounts. Thin-layer chromatography of organic urine extracts revealed four metabolites of HCCPD which were not chemically characterized.

Yu and Atallah (1981) also investigated the distribution of HCCPD in rats dosed with 25 mg/kg ^{14}C-HCCPD by gavage or 0.73 mg/kg by IV injection. Brain, heart, lung, muscle, fat, gonad, uterus, spleen, kidney, liver, blood, digestive system, skin, hair, and urinary bladder were analyzed for retained radiolabel at 8, 24, 48, or 72 hours after oral administration. In gavaged rats, the kidney contained 16.20 ppm HCCPD equivalents, whereas the liver retained 6.23 ppm, and the gonad, fat, lung, and blood retained between 1.28 and 1.89 ppm equivalents at 8 hours post-dosing. All other tissues had less than 1 ppm. At 24 and 48 hours post-dosing, the kidney and liver still had the highest concentrations of HCCPD equivalents.

Tissue concentrations were measured at 24 and 48 hours after IV administration (Yu and Atallah, 1981). Again, the kidney retained the highest concentration (2.64 ppm) of ^{14}C-HCCPD equivalents at 24 hours after administration. The blood, spleen and liver, in this order, contained the next highest concentrations. At 48 hours after IV administration, spleen and blood concentrations were the highest (about 2.95 ppm) and followed by the kidney at 2.02 ppm. All other tissues contained less than 0.42 ppm.

These data indicate that the tissue distribution of HCCPD and its metabolites was similar from 8 to 72 hours after oral administration, with HCCPD primarily retained in the kidney and liver. After IV administration, HCCPD and its metabolites were distributed primarily in the kidney, but the blood, spleen, and liver also had relatively high concentrations. The study shows that although the distribution of HCCPD and its metabolites varies somewhat with route of administration, the kidney and liver are the major organs of concentration for both oral and IV routes. When considering the tissue concentrations in proportion to the dose received, the data also indicate that HCCPD and its metabolites are retained longer after IV administration than after oral administration. The authors suggest that lower retention of orally dosed HCCPD is due to its poor absorption in the gut.

Based on blood data from IV dosed rats, Yu and Atallah (1981) developed an open two-compartment pharmacokinetic model. The model proposed that HCCPD was rapidly metabolized and distributed in the central compartment (blood, liver, kidney, and lung) and then gradually redistributed to the peripheral compartment (fat tissues) after IV injection. Comparison of observed to expected values for radiolabel concentration in blood showed a good agreement. Using the model, the authors predicted a biological half-life of 32 hours for HCCPD in the rat (Yu and Atallah, 1981). No modeling was performed for oral administration.

Lawrence and Dorough (1981) investigated differences in distribution between corn oil gavage and inhalation administration in female Sprague-Dawley rats. For inhalation studies, rats inhaled, via nose only exposure, 24 µg ^{14}C-HCCPD/kg (exposure concentration not reported) for single 1-hour periods. For measurable tissue levels of ^{14}C, the gavage dose had to be much higher, 6 mg/kg ^{14}C-HCCPD in 0.5 ml corn oil. Tissue samples were taken at 72 hours post-dosing, combusted and then $^{14}CO_2$ was trapped and counted. Radioactivity was measured in the trachea, lungs, liver, kidneys, and carcass. Levels were reported as a percentage of the administered radioactivity. After inhalation exposure, the carcass retained 7.8±2.0% of the dose, the lungs retained 2.0±0.4%, the kidneys retained 0.8±0.2%, and the liver retained 0.4±0.2%. After gavage, the carcass retained 1.87±1.16% of the dose, the kidneys retaining 0.47±0.06%, the liver retained 0.39±0.06% and other tissues retained less that 0.1% of the radiolabel. For either route, only trace amounts of radiolabel were found in fat.

In a similar study (Lawrence and Dorough, 1982), distribution of ^{14}C-HCCPD in female Sprague-Dawley rats was studied following oral, inhalation, and IV administration. Doses were 6 mg/kg via gavage, 24 µg/kg for the inhalation route (via nose cone), and 10 µg/kg for the IV route. Trachea, lungs, liver, kidneys, fat, and remaining carcass were assayed for $^{14}CO_2$ at 72 hours post-exposure. After inhalation exposure, the highest concentration of HCCPD equivalents was in the trachea (107±65.0 ppb), followed by lungs (71.5±55.2 ppb), and kidneys (29.5±20.2 ppb). After oral exposure, the highest concentrations were in the kidneys (3,272±84 ppb), liver (539±72 ppb) and lungs (420±250 ppb). Following IV exposure, the kidneys retained the highest concentration, 22.3±0.6 ppm, while the lungs retained 14.9±1.1 ppm, and the liver

retained 9.6±1.1 ppm HPCCD equivalents. The trachea retained only 3.3±1.7 ppm following IV administration. These data are consistent with those from Lawrence and Dorough (1981), showing that distribution depends upon route of administration, with oral and IV HCCPD resulting in generally similar distribution patterns. Oral and IV administration resulted in the highest concentrations of HCCPD equivalents in the kidneys and then in the liver and lungs, whereas inhalation exposure resulted in the highest concentrations in the trachea, followed by lungs and then kidneys. The concentration of HCCPD equivalents in fat was only appreciable for the oral route.

Results from a study of distribution of radiolabeled HCCPD by Dorough and Ranieri (1984) in rats and mice were consistent with those of Lawrence and Dorough (1982). Male and female Sprague-Dawley rats and mice were gavaged with 2.5 mg/kg or 25 mg/kg ^{14}C-HCCPD (in 0.9 ml corn oil for rats and 0.2-0.3 ml corn oil for mice). After both doses, the kidney contained the highest concentration of radiolabel in the rat, but the liver contained the highest concentration in the mouse at 1 and 7 days after exposure. A study of the distribution of dietary HCCPD was performed using concentrations of 1, 5, and 25 ppm in food (Dorough and Ranieri, 1984). After 15 days on the diet, radioassay of tissues collected from female rats showed the highest concentration of HCCPD equivalents/dietary ppm in the kidneys, fat, then in the gonads and liver at all dietary dose levels. Male rats retained the compound in the same distribution pattern as the female rats, but had higher concentrations of HCCPD equivalents/ppm diet in the liver than in the gonads. Female and male mice retained the compound primarily in the fat, then the liver, then the gonads and kidney. Gonads concentrated radioactive residues at a comparable, but slightly lower, level to fat in both species, whereas muscle and brain did not accumulate appreciable amounts, even at the 25 mg/kg dose.

Yu and Atallah (1981) also studied the nature of the metabolites in the tissues by extracting tissue homogenates with organic solvents. The majority of degradation products were polar and were organically extractable only after acidification. Attempts to identify the metabolites of HCCPD in rodents (Yu and Atallah, 1981; Mehendale, 1977; Shell, 1984) have been unsuccessful.

Yu and Atallah (1981) incubated fecal material from rats with aliquots of 315 µg of ^{14}C-labeled HCCPD to study the stability of HCCPD in this environment. Samples of the mixture were collected at 0, 1, 6, and 24 hours, homogenized and organically extracted. The remaining solid was dried and radioassayed, while the organic extracts were partitioned using an acetonitrile/water mixture, and the layers were radioassayed. The results indicated that the HCCPD was rapidly degraded in the feces with a half-life of 1.6 hours. The fact that antimicrobial compounds slowed the degradation indicated that microbial action was responsible for HCCPD breakdown in the fecal homogenate.

Samples taken from the contents of the duodenum, and small and large intestine from selected rats were homogenized and added to radiolabeled HCCPD in the presence or absence of antimicrobial agents (Yu and Attalah, 1981). Sampling and extraction proceeded as described for the fecal homogenates. The results of intestinal incubation indicated that HCCPD degradation proceeded slowly in the gut in a microbe-dependent fashion with a half-life of 10.1 hours. Degradation rates of HCCPD by liver homogenates was similar for active ($t_{1/2}$ = 14.2 hours) and denatured ($t_{1/2}$ = 12.4 hours) homogenates. Due to the similarity of degradation rates between active and denatured extracts, the authors proposed that the necessary cofactor(s) for proper liver enzyme activity to degrade HCCPD was likely not present in the prepared extracts, or that most of the degradation of HCCPD takes place outside of the liver.

El Dareer et al. (1983) performed *in vitro* binding experiments with HCCPD and varying biological materials obtained from rats to study the interaction of the compound with biological macromolecules. After an incubation of ^{14}C-HCCPD with the material for 0, 5, or 60 minutes, a series of organic extractions was performed. Liver homogenates, plasma, and whole blood incubated with the HCCPD formed virtually inextractable mixtures even at 0 minutes. Feces and intestinal contents, however, were easily extractable at 0 and 5 minutes and extractability did not decrease until the 60 minute incubation. The results show the high chemical reactivity of HCCPD toward biological materials.

3.3 Excretion

In the study by Mehendale (1977) which gavaged rats with ~ 6 mg/kg ^{14}C-HCCPD, urine and fecal samples were collected daily for 7 days. After 7 days, approximately 33% of the total radioactivity was excreted in the urine with 87% of that eliminated within the first 24 hours. Fecal excretion accounted for 10% of the administered dose with 60% of fecal excretion occurring during the first day. Only trace amounts of radioactivity were recovered in feces after the third day. Since individual tissues contained less than 0.5% of the radioactivity and only 43% had been excreted in feces and urine, Mehendale (1977) suggested that HCCPD may be eliminated, to a large extent, in exhaled air.

In another experiment, Mehendale (1977) injected ~ 6 mg/kg ^{14}C-HCCPD into the femoral veins of male rats and collected samples of blood and bile at 15, 30, 45, and 60 minutes. The radioactivity in blood decayed biexponentially with a terminal half-life of 1 hour. Approximately 9% of the radioactivity was excreted in bile over 1 hour. Pre-dosing the rats with 50 mg/kg/day HCCPD for 3 days by gavage had no effect on biliary excretion or on the decline of radioactivity in blood.

In the study by Yu and Atallah (1981), described in Section 3.1, urine and fecal samples were analyzed for radioactivity at 8, 24, or 48 hours after a single oral or IV dose of radiolabeled HCCPD. After gavage dosing, radiolabel was eliminated mainly in feces (70%) and in urine (17%) within 48 hours. Fecal excretion after oral administration was much greater than that observed by Mehendale (1977). When administered intravenously, the radioactivity was eliminated equally in feces (21%) and urine (18%) over the same time period.

Lawrence and Dorough (1981) administered 5 µg ^{14}C-HCCPD/kg to female rats via one-hour inhalation or by gavage to compare excretion by the two exposure routes. Urine and fecal samples were taken at 24, 48, and 72 hours post-dosing. Radioactivity in urine samples was counted in a scintillation counter, while fecal samples were combusted, and trapped $^{14}CO_2$ was assayed. At 24 hours after gavage, elimination was primarily in the feces (62.2±8.0%) as compared to the urine (22.8±1.8%). Fecal and urinary excretion after oral administration was similar to that observed by Yu and Atallah (1981). After inhalation exposure, elimination was

higher in urine (29.7±4.5%) than in feces (17.0±7.5%). The proportions of urine:fecal excretion did not change at 48 or 72 hours. Another inhalation experiment (Lawrence and Dorough, 1981) in which rats were administered 1.4-37.4 µg ^{14}C-HCCPD/kg, showed that excretion by exhalation was insignificant. Less than 1% of the radiolabel was eliminated as ^{14}C-HCCPD in expired air in the 24 hours following exposure and no $^{14}CO_2$ was detected in expired air.

A follow-up study by Lawrence and Dorough (1982) compared the fate of inhaled (24 µg/kg), oral (5 µg/kg), and IV (10 µg/kg) ^{14}C-HCCPD in female Sprague-Dawley rats. Radiolabeled residues were primarily excreted via the feces after oral and IV routes, and primarily via the urine following inhalation exposure. After three days, the percentage of the dose eliminated via the feces was significantly higher for oral administration (~70%) than it was for IV (~30%) or inhalation (~27%). These results for percentage urinary excretion confirm those of Yu and Atallah (1981) and Lawrence and Dorough (1981). Lawrence and Dorough (1982) found total body burden was much higher after IV dosing (31.0±7.8%), as compared to oral (2.8±1.1%) or inhalation (12.9±4.7%) exposure. Biliary excretion of label was found to be highest following oral exposures, accounting for 18% of the dose in 28 hours. Biliary excretion of ^{14}C-HCCPD was 13% of the IV dose and ~9% of the inhaled dose.

In the Dorough and Ranieri (1984) study, female rats and mice intubated with a single low (2.5 mg/kg) dose of radiolabeled HCCPD excreted the majority of the label in feces as compared to urine at both one and seven days post-dosing. After one day, rats excreted 65.2% of the dose in feces and 12.4% in urine while mice excreted 42.1% of the dose in feces and 13.8% in urine. The percentage excretion was higher at 7 days with a similar feces:urine ratio. At 25 mg/kg, there were no appreciable differences between rats and mice in the amount of radioactivity excreted in feces vs. urine. Results from males rats treated with 25 mg/kg ^{14}C-HCCPD showed that excretion in male rats was also similar to that in females. Fecal excretion after three days was 73.6% of the administered dose while urinary excretion was 13.4%.

El Dareer et al. (1983) also investigated the disposition of ^{14}C-HCCPD administered to rats via a single oral gavage dose (4.1 mg/kg or 61 mg/kg ^{14}C-HCCPD) in 1 ml corn oil/150 g body weight, a single IV dose (0.59 mg/kg ^{14}C-HCCPD) in 0.15 ml 1:1:4 Emulphor EL-

620:ethanol:water/150 g body weight, or a single inhaled dose (1.1 mg administered over 2 hours via whole body exposure). Following oral doses, >90% of the radioactivity was excreted after 72 hours, with twice as much contained in the feces as in urine. Only 34% of the IV dose was excreted in the feces after 72 hours, with urinary excretion accounting for 15.8%, and 39.0% remaining in the tissues. At 6 hours following the inhalation exposure, excretion was primarily via the urine (41.0% of dose). The amount excreted via the feces (28.7%) was comparable to that remaining in tissues (28.9%). At 72 hours after inhalation, excretion was roughly equal between feces and urine (40–50%), with only a small portion remaining in tissues (11%). El Dareer et al. (1983) essentially confirms the results for urinary and fecal excretion obtained by Lawrence and Dorough (1982) for oral, IV, and inhalation routes of exposure.

Another study investigated the excretion of HCCPD in rats, rabbits and mice after the administration of 20 mg/kg radiolabeled HCCPD (Shell, 1984). Rats and mice were dosed via gavage (2 ml corn oil/kg body weight), while rabbits were dosed via gelatin capsule. Consistent with the results of previous investigators (Yu and Atallah, 1981; Lawrence and Dorough, 1982; Lawrence and Dorough, 1982; Dorough and Ranieri, 1984), fecal excretion of radiolabel was predominant, with urinary excretion secondary. By the end of three days, 85-92% of the entire dose was eliminated. Urinary excretion was 20%, 23% and 35% of the administered radiolabel for rats, mice and rabbits, respectively. Fecal excretion over the same period was 68%, 69% and 51% for rats, mice and rabbits, respectively. As also shown by Lawrence and Dorough (1981), little or no $^{14}CO_2$ was detected in expired air (measured for rats only). After IV administration of 24 mg/kg radiolabeled HCCPD (200 mg/ml in 30 µl ethanol) to a separate group of rats, an equal percentage of the dose administered was excreted in the feces (10%) and urine (9%), with much less of the total dose excreted (19%) at the end of three days. The equal proportions of fecal vs urinary excretion was similar to other studies using IV administration, but the percentage of the total dose excreted was much less than that found in other studies (Yu and Atallah, 1981; Lawrence and Dorough, 1982; El Dareer, 1983).

The majority of these metabolism studies indicate that excretion of HCCPD metabolites varies depending on exposure route. Fecal excretion predominates after oral exposure, while

urinary excretion predominates following inhalation exposure. Microbial metabolism to polar metabolites in the gut is likely to be responsible for the large proportion of fecal excretion after oral administration. Fecal and urinary excretion are approximately equal after IV administration. HCCPD metabolites produced following inhalation exposure are retained in the bodies of rodents longer than those from ingested HCCPD, which may indicate that the metabolism to polar compounds occurs more slowly after inhalation exposure.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, AND CLINICAL CONTROLS

4.1.1. Buncher, C.R., Moomaw, C, Sirkoski, E. 1980. Mortality study of Montague plant. Unpublished report for Hooker Chemical Corporation. Doc. # 878212111. NTIS/OTS84003A.

Buncher et al. (1980) conducted an occupational mortality study with 341 workers at the Hooker Chemical Corporation plant in Montague, MI. The plant produced HCCPD and other chlorinated hydrocarbons. Three hundred forty-one employees who had worked at least 90 days between October 1, 1953 and December 31, 1974 were included in the cohort. Follow-up was through December 31, 1978. Expected deaths were determined using sex-, age- and year-specific U.S. mortality rates. The 24 deaths grouped in such causal categories as all causes, all cancers, diseases of the circulatory system, diseases of the digestive system, and external causes were fewer than expected. The six observed cancer deaths included one cancer each in the esophagus, large intestine, breast, and kidney, and two of the respiratory system. The authors indicate that the ratio of observed to expected deaths for the respiratory cancers (0.87) and colon cancer (1.75) are not statistically unusual. The remaining cancers have ratios greater than or equal to 5; however, the small numbers of deaths prevent drawing a firm conclusion. The short follow-up period is also a limitation in this study.

4.1.2. Wang, H.H., MacMahon, B. 1979. Mortality of workers employed in the manufacture of chlordane and heptachlor. J Occup Med 21:745-748.

This retrospective mortality study involved white male workers from the Velsicol Chemical Plants in Marshall, IL, and Memphis, TN. The population studied consisted of 1403 white males currently or formerly employed for more than 3 months during the years 1946–1975 for the IL plant and 1952-1976 for the TN plant. The plants manufactured heptachlor, and chlordane, for which HCCPD is an intermediate, during those periods. Approximately 34% of the subjects had less than 10 years follow-up and 36% had 20 or more years follow-up. Expected deaths for these person-years were calculated from white male national mortality rates through 1975. Observed deaths due to all causes were significantly lower than expected deaths. Deaths due to cerebrovascular disease, however, were significantly elevated over those expected. Because exposure to several organochlorines occurred, the increase in cerebrovascular disease could not be attributed to HCCPD exposure. Deaths due to all cancers were less than expected, but deaths due to lung cancer were greater than expected, although not significantly. Lung cancer deaths were not associated with duration of employment or duration of follow-up, but the numbers available for such analysis were small. No data on cigarette smoking were available for this study group. There was one death each from cancer of the liver, bladder, prostate, and central nervous system.

4.1.3. Shindell and Associates. 1980. Report of epidemiologic study of the employees of Velsicol Chemical Corporation plant, Marshall, Illinois, January 1946–December 1979. Unpublished report for Velsicol Chemical Corporation, July 1980. Doc. # 40-8149074.

Shindell and Associates (1980) conducted a mortality study of 783 workers employed at least 3 months between January 1, 1946 and December 31, 1979 at the Velsicol Chemical Corporation plant in Marshall, IL. The aim of the study was to evaluate the overall health status of all former and current employees with three months or more employment during a time when the Marshall plant was manufacturing chlordane. This cohort is similar to that studied by Wang and MacMahon (1979), but included non-white males and women. The cohort included 783

individuals comprised of 689 white males, 10 non-white males, and 84 females. The two studies employed different follow-up techniques. The vital status of 97.4% of the cohort was known. The causes of death examined included all deaths, malignant neoplasms, diseases of the heart and circulatory system, cerebrovascular disease, trauma, and others. The number of observed deaths in each category was compared to the number of expected deaths calculated from race- and sex-specific U.S. mortality rates for appropriate 5-year periods. No excess deaths related to any specific job class or product were seen. Except for "other deaths" in females, the number of deaths observed were lower than the number expected. The 22 deaths from cancer included brain, kidney, liver, lung, and digestive system cancers. Eight of the 22 cancer deaths were from lung cancer. The number of expected deaths for each of these specific cancers was not calculated. This study reported no significant differences between mortality of plant employees and individuals from the U.S. population matched for race, age, and sex, during the time period the cohort was studied. The authors noted the healthy worker effect in mortality data from the Marshall plant.

4.1.4. Shindell and Associates. 1981. Report of epidemiologic study of the employees of Velsicol Chemical Corporation plant, Memphis, Tennessee, January 1952–December 1979. Unpublished report for Velsicol Chemical Corporation, March 1981. Doc. # 40-8149074.

The second mortality study performed by Shindell and Associates involved the Velsicol plant in Memphis, TN. The cohort included 1115 employees with a minimum of 3 months of employment between January, 1952 and December 31, 1979. The purpose of the study was to evaluate the overall health status of all former and current employees with three months or more employment during a time when the plant was manufacturing heptachlor. The study design was the same as Shindell and Associates (1980). The vital status of 92.8% of the cohort was known. Consistent with the earlier Shindell study, this investigation revealed no significant differences between mortality of plant employees and the overall U.S. population. Deaths from strokes and from trauma showed an insignificant increase over the number of expected deaths. The distribution of the standard mortality ratio of deaths by site of cancer and job class showed a

non-significant excess of lung cancer in maintenance workers. The authors concluded that there was no pattern of neoplasia suggestive of job-related risk. In addition, mortality by cause was consistent regardless of tenure of employment at other plants.

4.1.5. Brown, DP; Ditraglia, D; Namekata, T; et al. 1980. Mortality study of workers employed at organochlorine pesticide manufacturing plants. U.S. Dept of Health, Education and Welfare and University of Illinois. Unpublished report. May, 1980. Doc. # 40-8149074

This mortality study involved cohorts from four different chemical plants that manufactured organochlorine pesticides. The cohorts were defined as all workers at each plant who had worked at least 6 months prior to December 31, 1964. Causes of deaths which occurred prior to December 31, 1976 were recorded. The entire study included about 2100 individuals, but the cohorts at each plant were evaluated separately. These cohorts overlapped the one used in the Wang and MacMahon study (1979), but extended the follow-up period. Observed deaths in the cohorts were far fewer than expected, reflecting the healthy worker effect. The expected value was calculated using U.S. white-male cause-specific mortality rates, but the report did not specify the sex or ethnicity of the employees. The increase in cerebrovascular disease, observed in the Wang and MacMahon study (1979), was not reported in this study. A decrease in expected deaths from all malignant neoplasms in each plant was observed, but it was not statistically significant. There were slight, but not statistically significant, increases in stomach cancer deaths in one plant, and slight excesses of cancers of the esophagus, rectum, liver, and lymphatic and hematopoietic systems in another plant. Exposure to multiple organochlorine compounds in each of the plants precludes linking these cancer cases with exposure to HCCPD or any other individual compound.

4.1.6. Kominsky, J.R., Wisseman, III, C.L., Morse, D.L. 1980. Hexachlorocyclopentadiene contamination of a municipal wastewater treatment plant. Am Ind Hyg Assoc J 41:552-556.

This report documents an accidental acute occupational exposure to high concentrations of HCCPD when an unidentified odoriferous and viscous substance accumulated on the bar screens and grit collection systems of a wastewater treatment plant. When employees used steam to remove the substance, a blue haze was generated and permeated the primary water treatment area, forcing approximately 20 workers to seek medical attention for tracheobronchial irritation. On the following day, after a heavy rain, personnel noticed a similar blue haze over the grit collection channels accompanied by an offensive odor throughout the primary treatment area. The plant was closed two days later when HCCPD and octachlorocyclopentene (OCCP) were detected in the wastewater. Airborne concentrations of HCCPD and OCCP during the exposure period were not known, but four days after the plant was closed for cleaning, concentrations in the screen and grit chambers were 270-970 ppb, and HCCPD concentrations in the blue haze were as high as 19,200 ppb (217 mg/m^3)[1].

Of the 177 treatment plant employees (23 females, 154 males) who responded to a medical questionnaire, 59% reported symptoms of eye irritation, 45% reported headaches, and 27% reported throat irritation. Six weeks after exposure to the organochlorines, many complaints of persistent health effects were reported: headache (18%), persistent fatigue (15%), chest discomfort (13%), skin irritation (10%), and cough (9%). A review of the medical records of 90 employees who were observed by the plant physician over a 2-month period starting with the first reports of contamination revealed symptoms of headache, and mucous membrane and respiratory tract irritation. Unusual symptoms were reported by individuals with acute, high-level exposure to the compound**s**, including one report of “burning feet” (the individual’s boots deteriorated in contaminated sludge), three incidences of “sunburn-like” facial irritation, seven reports of rashes on exposed skin, and seven reports of transient confusion or memory loss. No changes were

[1]Calculated using conversion of 1000 ppb = 11.3 mg/m^3.

observed for the 28 employees who received chest x-rays. Arterial blood gas analyses were performed for 16 of the 28 employees and pulmonary function tests were performed for 22 people. Neither test reveal abnormalities.

Laboratory tests from 97 cleanup crew members revealed no significant abnormalities in renal function, complete blood counts, or urinalyses, however, 18 cleanup workers had mild liver function abnormalities exhibited by abnormal serum values in glutamate-oxalacetate transaminase, alkaline phosphatase, total bilirubin, and/or lactate dehydrogenase. The proportion of the 18 workers that underwent pre-exposure monitoring is uncertain since the authors indicate only that 52 of the 97 cleanup workers were monitored prior to exposure. Thus, the relationship of the abnormal liver indices to exposure is uncertain. However, seven persons did have increased serum glutamate-oxalacetate transaminase that seemed to be temporally related to exposure to contaminated sewage. The authors concluded that exposures to HCCPD and association compounds may produce liver damage. The association of HCCPD exposure and liver function abnormalities are confounded, however, by the lack of information on pre-exposure monitoring and co-exposure to OCCP.

4.1.7. Boogaard, P.J., Rocchi, P.S.J., van Sittert, N.J. 1993. Effects of exposure to low concentrations of chlorinated hydrocarbons on the kidney and liver of industrial workers. Br J Ind Med 50:331-339.

In this study, 73 male operators in a chemical plant that produced several different chlorinated hydrocarbons were evaluated for liver and kidney toxicity. The subjects were employed for an average of 8.2 years (0.5–23 years). A control group consisted of 35 male employees who were not occupationally exposed to the chemicals. The control group was well-matched to the exposed population in all selected parameters except age. Age was a confounding factor for several of the biochemical analyses performed.

Exposure to HCCPD, allyl chloride, 1,3-dichloropropene, and epichlorohydrin was measured by personal samplers on a few individuals. While concentrations of 1,3-dichloropropene and epichlorohydrin were well below the applicable occupational exposure

standards (5 and 4 mg/m^3, respectively), exposures to allyl chloride and occasionally exceeded the maximum allowable concentrations of 3 and 0.11 mg/m^3 (0.01 ppm), respectively. Individual exposures could not be estimated because personal samplers were used on few employees.

Biochemical analyses indicated no differences between the control and exposed populations on any of the liver function tests (serum alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, gamma-glutamyltranspeptidase, lactate dehydrogenase, and total serum bile acids). Further, no statistically significant differences were observed in kidney function tests measuring urinary levels of alanine aminopeptidase, N–acetyl-β-D-glucosaminidase, retinol binding protein, albumin and total protein. The exposed group had greater urinary albumin levels than controls (8.09 mg/g vs. 4.68 mg/g), but the difference was not statistically significant. These results indicate that exposure to occupational concentrations of these chlorinated hydrocarbons does not cause significant liver or kidney damage. However, the lack of definitive exposure data, and the simultaneous exposure to other chemicals, does not allow the prediction of toxicity solely from HCCPD.

4.2. SUBCHRONIC/CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS-INHALATION AND ORAL

4.2.1. Inhalation Studies

4.2.1.1. NTP. 1994. Toxicology and carcinogenesis studies of hexachlorocyclopentadiene in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program Technical Report Series 437:318.

In 13-week range-finding studies in F344/N rats and B6C3F1 mice, NTP exposed groups of animals (10 per sex per species) for 5 days per week, 6 hours per day, to atmospheres containing 0, 0.04, 0.15, 0.4, 1, or 2 ppm HCCPD (0, 0.45, 1.7, 4.5, 11, or 22 mg/m^3, respectively). Standard bioassay data including body weights, clinical chemistry, hematology, urine analysis, organ weights, pathology, and histopathology were collected. All rats in the 11 and 22 mg/m^3 groups died within 4 weeks. Clinical effects in rats included listlessness in the 22 mg/m^3 group from week 1, in the 11 mg/m^3 group from week 2, and in the 4.5 mg/m^3 group

during week 3. Rats in the 11 and 22 mg/m^3 groups also experienced respiratory distress (mouth breathing and increased respiration rate). Male rats in the 4.5 mg/m^3 dose group exhibited a statistically significant decrease in body weight compared to controls, but it is not considered to be toxicologically significant since it was less than 10%. Body weights of treated female rats were similar to controls. No other treatment-related clinical findings of toxicity were reported.

Necropsy of rats in the 11 and 22 mg/m^3 groups revealed extensive coagulation necrosis in the respiratory epithelium of the nose, larynx, trachea, bronchi and bronchioles. Necrosis was accompanied by inflammatory signs such as vascular congestion, edema, fibrin accumulation, and neutrophil and mononuclear cell infiltration. Male rats in the 4.5 mg/m^3 group exhibited necrotizing and suppurative inflammation of the nose, bronchus, and bronchioles and squamous metaplasia of the nose as well as increased lung weights. The squamous metaplasia was focal in nature, generally observed on the tips of the turbinates, and characterized by stratification of the epithelium to form three to four poorly defined layers of flattened, nonkeratinized polygonal cells. Female rats seemed to be less sensitive. At the 4.5 mg/m^3 exposure, the only nose effects were suppurative inflammation and fewer females than males exhibited necrotizing and suppurative inflammation of the bronchus and bronchioles. Since no respiratory lesions were seen at exposures lower than 4.5 mg/m^3 HCCPD, the NOAEL was 1.7 mg/m^3 and the LOAEL was 4.5 mg/m^3 HCCPD.

All mice in the 11 and 22 mg/m^3 groups died within five weeks. Before the end of the study, seven deaths occurred in the 4.5 mg/m^3 group, one death occurred in the 1.7 mg/m^3 group, and three deaths occurred in the 0.45 mg/m^3 group. Six deaths in the female control group were attributed to a defective feeder. Clinical effects included listlessness in the 4.5 mg/m^3 and 11 mg/m^3 groups. No chemical-related differences in hematology, clinical chemistry, or urinalysis parameters were reported in exposed males or females. Males in the 0.45 mg/m^3 group exhibited a statistically significant decrease in weight which was not toxicologically significant (i.e., <10%). Body weights of exposed animals were similar to controls in all other groups.

As evidence by a somewhat lower frequency of effects, mice were not as sensitive to the respiratory toxicity of HCCPD as rats. Male mice exhibited significant increases in suppurative

inflammation of the nose and squamous metaplasia of the trachea at 4.5 and 11 mg/m^3, and acute necrosis and suppurative inflammation of the nose, acute necrosis of the larynx, trachea, and lung, and congestion of the lung at 22 mg/m^3. Female mice had serous inflammation of the nose at 4.5 mg/m^3, and suppurative inflammation of the nose, squamous metaplasia of the larynx and trachea, and necrotizing inflammation of the lung at 11 mg/m^3. At the highest dose, female mice presented the same spectrum of effects as male mice. Since no effects were observed in mice at 1.7 mg/m^3, the NOAEL was 1.7 mg/m^3 and the LOAEL was 4.5 mg/m^3.

4.2.1.2. Rand, G.M., Nees, P.O., Calo, C.J., Alexander, D.J., and Clark, G.C. 1982a. Effects of inhalation exposure to hexachlorocyclopentadiene on rats and monkeys. J Toxicol Environ Health 9:743-760.

Rand, G.M., Nees, P.O., Calo, C.J., Clark, G.C., and Edmondson, N.A. 1982b. The Clara cell: An electron microscopy examination of the terminal bronchioles of rats and monkeys following inhalation of hexachlorocyclopentadiene. J Toxicol Environ Health 10:59-72.

In these studies, Sprague-Dawley rats and cynomolgus monkeys inhaled, via whole body exposure, 97.7% pure HCCPD at 0, 0.01, 0.05 or 0.20 ppm (0, 0.11, 0.56, or 2.3 mg/m^3, respectively)[2] for 6 hours/day, 5 days/week, for 14 weeks. Each exposure group contained 40 male and 40 female rats, and six male and six female monkeys. To investigate the Clara cell of the lung as a potential target for HCCPD toxicity, Rand et al. (1982b) performed electron microscopy upon lung cell preparations from three rats of each sex and three monkeys of each sex.

Rand et al. (1982a) reported no mortalities or adverse clinical signs in monkeys at any exposure level. Body weight gain and food consumption were not significantly different between groups. Pulmonary function tests (blood gas analysis, lung mechanics, lung ventilation) were normal. No eye lesions were noted, and no exposure-related changes were noted in hematology, clinical chemistry, urinalysis, organ weights, macroscopic pathology, and histopathology. One

[2]Calculated using conversion of 1 ppm = 11.3 mg/m^3.

male monkey from the 2.3 mg/m^3 group exhibited occasional Clara cells containing "electron-lucent inclusions in the apex and base of the cell, surrounded by a single limiting membrane." Since the electron-lucent inclusions have no known relationship to pathology, the existence of the inclusions in the Clara cells was not considered to be adverse. Since no adverse effects were noted, the NOAEL for monkeys was 2.3 mg/m^3 HCCPD.

Rand et al. (1982a) reported that four rats from three exposure groups, including the control group, died or were killed due to severe illness, but illness was not attributed to HCCPD exposure. The only significant clinical sign reported in male rats was dark, red eyes observed in the 0.56 and 2.3 mg/m^3 dose groups. This effect, which was first noted after the 10th exposure and disappeared after the 20th exposure, was also noted in a range-finding study performed by the same authors, and was considered to be related to HCCPD exposure. Ophthalmoscopic examination revealed no eye lesions. There were no exposure-related changes in body weight gain, food or water consumption, or urinalysis. After 12 weeks of exposure, there were slight, occasionally statistically significant increases in hemoglobin, red blood cell count, and mean corpuscular hemoglobin concentration with a corresponding reduction in the mean cell volume in males at 0.11 and 2.3 mg/m^3 and in females at 0.56 and 2.3 mg/m^3. The authors observed similar effects in a range-finding study and considered them to be indicative of impaired respiratory function. There were no other effects on hematology. Statistically significant decreases in mean liver weight occurred in all treatment groups and in kidneys of all treated males after 13 weeks of exposure. The Clara cells of all treated rats contained a statistically significant increase in the number of the electron-lucent inclusions as compared to controls (Rand et al., 1982b). No treatment related gross pathology or histopathology was observed. Since the changes in hematologic parameters were not dose-related, the kidney and liver weight changes were not accompanied by pathology, and the Clara cell inclusions were not related to pathology, the NOAEL for rats was 2.3 mg/m^3. There was no LOAEL.

4.2.1.3. Clark, D.G., Pilcher, A., Blair, D., Martin, J.G., Hendy, R., Wiggins, D., and Brown, V.K.H. 1982. Thirty week chronic inhalation study of hexachlorocyclopentadiene

(HEX) in rats. Group Research Report SBGR.82.051. NTIS/OTIS43022.

In a 30-week study, Wistar rats inhaled 0, 0.05, 0.1 or 0.5 ppm (0, 0.56, 1.1, or 5.6 mg/m^3, respectively)[3] HCCPD in inhalation chambers for 6 hours/day, 5 days/week, followed by a recovery period of 14 weeks free from exposure. Chemical purity of the compound decreased from 96% to 90% during the course of the study due to oxidation. Clinical signs included sneezing and lethargy in animals exposed to 5.6 mg/m^3 throughout the study. Four males and two females from this group died during exposure. Pathological analyses revealed that the animals that died prematurely had signs of bronchopneumonia. Two of the deceased rats had enlarged adrenals and the thorax contained watery or bloodstained fluid. No deaths or clinical signs of toxicity were reported in the other exposure groups.

Males in the 1.1 mg/m^3 and 5.6 mg/m^3 groups had significantly higher mean erythrocyte counts, hemoglobin concentrations, hematocrit and absolute numbers of neutrophils, and significantly lower lymphocyte counts than the controls. Mean absolute numbers of lymphocytes were lower in females at the 5.6 mg/m^3 dose.

Body weights of males from the 5.6 mg/m^3 dose group were significantly lower than controls from the seventh week until the end of the study, but, at 6% less than controls, were not toxicologically significant. Several increases in body weights in females exposed to HCCPD, compared to controls, were noted in the first half of exposure. At the end of the exposure period, body weights of the 1.1 mg/m^3 and 5.6 mg/m^3 females were similar to controls, however, at the end of the recovery period, body weights of those groups were less than controls by 11% and 9%, respectively. Kidney weights were significantly increased in females in the 5.6 mg/m^3 group after exposure for 30 weeks. Male heart weights were decreased at 30 weeks in the 5.6 mg/m^3 group and male spleen weights were decreased at 44 weeks in the 0.56 and 1.1 mg/m^3 groups. Testes weights were significantly increased at 44 weeks in the 5.6 mg/m^3 group. The organ weight effects were not considered to be biologically significant by the study authors.

[3]Calculated using conversion of 1 ppm = 11.3 mg/m^3.

Rats at the 5.6 mg/m^3 dose showed pulmonary degenerative changes including epithelial hyperplasia, edema, and sloughing of the bronchiolar epithelium in both sexes and epithelial ulceration and necrosis in the males. No degenerative changes in the lungs were observed in the 0.56 or 1.1 mg/m^3 dose groups. Rats in the 5.6 mg/m^3 group also had mild degenerative changes in the liver and kidney. The authors suggested that the toxic action of HCCPD involves an extreme local irritation of the respiratory tract that causes death by respiratory failure following bronchopneumonia. The authors considered that the mild degenerative changes in the livers and kidneys of a few rats were unlikely to contribute significantly to HCCPD's toxicity in the rat. The results of this study indicate a NOAEL of 1.1 mg/m^3 and a LOAEL of 5.6 mg/m^3 for the critical effect of respiratory tract histopathology. Correction for the HCCPD content of the administered compound (90%) gives a NOAEL of 1 mg/m^3 and a LOAEL of 5 mg/m^3.

4.2.1.4. NTP. 1994. Toxicology and carcinogenesis studies of hexachlorocyclopentadiene in F344/N rats and B6C3F1 mice (inhalation studies). National Toxicology Program Technical Report Series 437:318.

The National Toxicology Program conducted 2-year inhalation exposure studies in F344/N rats and B6C3F1 mice. Groups of 60 animals per sex per species were exposed for 5 days per week, 6 hours per day, to atmospheres containing 0, 0.01, 0.05, or 0.2 ppm (0, 0.11, 0.56, or 2.23 mg/m^3, respectively) HCCPD. Ten male and 10 female rats and mice from each exposure group were evaluated at 15 months. Standard bioassay data including body weights, urinalysis, organ weights, pathology, and histopathology were collected. Monitoring the stability of the compound throughout the study, showed that no degradation took place for up to 2 years.

Exposure to HCCPD did not significantly effect survival of rats or mice, but the decrease in survival of female mice approached statistical significance in the 2.23 mg/m^3 group due to suppurative inflammation of the ovary. Body weights of rats were unchanged by HCCPD exposure, but body weights of male and female mice were reduced in the 2.23 mg/m^3 group.

Neoplastic lesions: No exposure-related increases in neoplasms were seen in male or female rats or mice. Male rats in the 2.23 mg/m^3 group, however, exhibited a significant increase

in the incidence of pars distalis adenoma of the pituitary (66%). Since the historical control incidence of pars distalis adenoma in male F344/N rats from other NTP inhalation studies was 60%, NTP considered this tumor to be unrelated to HCCPD exposure. NTP concluded that HCCPD exhibited "no evidence of carcinogenic activity" (NTP, 1994)

Non-neoplastic lesions: In female rats, significant increases in incidence of squamous metaplasia of the larynx were seen in the 0.11 and 2.23 mg/m^3 groups, but not in the 0.56 mg/m^3 group (see Table 1 for incidence). The lesion, described as stratified squamous epithelium several cell layers thick in areas usually lined by columnar epithelium, was considered to be of minimal severity in all groups. Because there is individual variation in the location of the transition between squamous and columnar epithelium and in obtaining consistent tissue sections in the treated rats, NTP indicated that the significance of this metaplasia is unknown. In

Table 1. Incidence[a] of selected respiratory tract lesions in rats from NTP (1994)

	Males				Females			
Lesion	0 mg/m^3	0.11 mg/m^3	0.56 mg/m^3	2.23 mg/m^3	0 mg/m^3	0.11 mg/m^3	0.56 mg/m^3	2.23 mg/m^3
Nose pigmentation	1/48	46/50	48/49	48/50	0/50	34/50	47/49	48/50
Trachea pigmentation	0/48	0/50	0/48	5/50	0/50	0/50	0/49	1/50
Lung pigmentation								
Bronchiole	0/50	0/50	0/50	49/50	0/50	25/50	42/49	50/50
Peribronchiole	0/50	0/50	2/50	16/50	3/50	1/50	4/50	2750
Squamous metaplasia of larynx	NR	NR	NR	NR	9/50	20/50	15/48	24/50

[a] Compared to number examined.
NR-not reported.

addition, a dose-response relationship was not evident. Exposure-related increases in yellow-brown granular pigmentation within the cytoplasm of epithelial cells of the nose, trachea, and

lung were also observed in both sexes of rats; however, the pathological significance of this effect is unknown.

Exposure-related increases in pigmentation of the respiratory epithelium of the nose, trachea, and lung were also seen in male and female mice (see Table 2 for incidence). Female mice also exhibited a dose-related increase in the incidence of suppurative ovarian inflammation that was significantly different from controls at 0.56 and 2.23 mg/m^3 HCCPD. The lesion was similar to other utero-ovarian infections observed in mice in NTP studies and is apparently caused by Klebsiella infections. At 2.23 mg/m^3 HCCPD, increases in suppurative inflammation of the nose were noted in both male and female mice during the interim evaluation at 15 months and at study termination. In the 13 week study, this effect was noted in males at 4.5 mg/m^3 and in females at 11 mg/m^3 HCCPD.

Table 2. Incidence[a] of selected respiratory tract lesions in mice from NTP (1994)

	Males				Females			
Lesion	0 mg/m^3	0.11 mg/m^3	0.56 mg/m^3	2.23 mg/m^3	0 mg/m^3	0.11 mg/m^3	0.56 mg/m^3	2.23 mg/m^3
Nose								
Pigmentation	0/50	45/50	50/50	44/50	0/49	40/50	48/50	41/48
Suppurative inflammation	0/50	0/50	1/50	36/50	4/49	0/50	3/50	40/48
Trachea pigmentation	0/50	29/50	48/50	48/50	0/49	6/50	43/48	42/47
Lung pigmentation	0/49	2/50	42/50	45/50	0/48	0/50	27/50	44/49
Suppurative ovarian inflammation	NA	NA	NA	NA	0/49	3/50	6/50	17/50

[a] Compared to number examined.
NA-not applicable.

Necrotizing inflammation of the bronchus/bronchioles, a response observed in NTP's subchronic study, was not reported in the 2-year study in rats or mice. Because rats exhibited no

exposure-related pathology or histopathology, the NOAEL for rats was 2.23 mg/m^3 HCCPD, the maximum exposure concentration. The NOAEL for mice was 0.56 mg/m^3 and the LOAEL was 2.23 mg/m^3 based on increased incidence of suppurative inflammation of the nose of both sexes. Suppurative ovarian inflammation was not considered to be the critical effect since it was attributed to Klebsiella infection.

4.2.2. Oral Studies

4.2.2.1. Abdo, K.M., Montgomery, C.A., Kluwe, W.M., Farnell, D.R., and Prejean, J.D. 1984. Toxicity of hexachlorocyclopentadiene: Subchronic (13-week) administration by gavage to F344 rats and B6C3F1 mice. J Appl Toxicol 4:75-81.

This subchronic study investigated the systemic toxicity of HCCPD given by gavage to weanling F344 rats and B6C3F1 mice. HCCPD (97.4% pure) was dissolved in corn oil and administered daily, 5 days per week, for 13 weeks. Ten rats/sex/dose received 0, 10, 19, 38, 75, or 150 mg/kg HCCPD. Ten mice/sex/dose received 0, 19, 38, 75, 150 or 300 mg/kg HCCPD. Stability of the gavage mixture, or the frequency of preparation, was not reported. Although data on clinical signs, body weights, organ weights, gross pathology, and histopathology were collected, no clinical chemistry, hematology, or urine analysis was performed as required by current test guidelines (U.S. EPA, 1998b).

Table 3 shows the mortality rates for rats and mice. The deaths of six male rats in the 150 mg/kg group, and one in the 75 mg/kg group, were attributed to HCCPD. All male mice and three females in the 300 mg/kg group died before the end of the study. Other premature deaths in treated rodents were attributed to gavage error. Clinical signs of ruffled fur and slight inactivity were noted in both rats and mice in the two highest dose groups. Significant body weight decreases (i.e., $\geq$10% less than controls) were noted in male rats in the 38, 75, and 150 mg/kg groups and in female rats in the 75 and 150 mg/kg groups. In mice, significant decreases in body weight were noted in males in the 150 mg/kg group and in females in the 300 mg/kg group. Data from organ weight ratios were significantly greater than controls for female rats at 75 and 150 mg/kg for right kidney:brain and at 38, 75, and 150 mg/kg for liver:brain. Liver:brain and right

kidney:brain weight ratios were significantly increased compared to controls at all doses in female mice. In addition, the lungs: brain ratio was significantly elevated over controls at the highest dose in female mice. Organ weight ratios were unaffected in male mice.

Table 3. Mortality for mice and rats (Abdo et al. , 1984)

Dose (mg/kg)	Male Rats	Female Rats	Male Mice	Female Mice
0	3/10	1/10	1/10	0/10
10	1/10	2/10	-	-
19	1/10	2/10	0/10	0/10
38	1/10	1/10	0/10	0/10
75	3/10	3/10	0/10	0/10
150	7/10	5/10	0/10	0/10
300	-	-	10/10	3/10

Necropsy revealed grossly observed lesions detected in the gastric mucosa in both rats and mice. These lesions consisted of black discolored foci, red cysts and ulceration in rats gavaged with 75 and 150 mg/kg HCCPD. Thickening of the mucosa was also observed in mice in the 150 and 300 mg/kg groups. Histopathological analyses noted forestomach lesions that ranged from minimal to marked in severity and were focal to diffuse in distribution. Notable features were hyperplasia, acanthosis, and hyperkeratosis of the epithelial surface of the forestomach and increased mitotic activity in the basal layer of the epithelium. Forestomach lesions were only discernible at and above the 38 mg/kg dose in male rats, but were seen (identified as epithelial hyperplasia and focal inflammation) in female rats at the 19 mg/kg dose (see Table 4). Forestomach lesions were noted in male and female mice at the 38 mg/kg dose (see Table 5). The forestomach lesions are believed to be a manifestation of irritation which is consistent with the observation of dermal irritation (Treon et al., 1955; Industrial Biotest

Laboratories, 1975a; HEW, 1978;) and other portal of entry effects from HCCPD exposure (Clark et al., 1982 and NTP, 1994). No forestomach lesions were observed in control rodents of either species.

Toxic nephrosis of the kidney was observed in male and female rats in the 38, 75, and 150 mg/kg groups, and in female mice in the 75, 150, and 300 mg/kg groups (see Tables 4 and 5). The incidence was not dose-related.

Table 4. Incidence[a] of stomach and kidney lesions in rats from Abdo et al. (1984)

	Males						Females					
Dose (mg/kg) Lesion	0	10	19	38	75	150	0	10	19	38	75	150
Stomach Lesions	0/10	0/10	0/10	5/10	9/10	8/9	0/10	0/10	2/10	5/10	9/10	9/10
Toxic Nephrosis	0/10	0/10	0/10	10/10	9/10	8/10	0/10	0/10	0/10	10/10	10/10	10/10

[a] Compared to total number of animals examined.

The kidney lesions were predominantly limited to the terminal portion of the proximal convoluted tubules in the inner cortex and were characterized by dilated tubules and epithelial changes consisting of cytomegaly, karyomegaly, and anisokaryosis with nuclear and cytoplasmic vacuolization. Acute tubular necrosis, which was morphologically distinct from the toxic nephrosis, was observed in 7 of the 10 male mice in the 300 mg/kg group, and may have caused the early mortality in this group. Although histopathologic changes in mice did not occur at doses below 38 mg/kg HCCPD, liver weights increased in a dose-dependent fashion starting at 19 mg/kg HCCPD. Because organ weight changes occurred only in females of both rodent species, and toxic nephrosis was not observed in male mice, this report indicates that female rodents may be generally more susceptible to the adverse effects of ingested HCCPD to the kidney and liver.

Based on the irritant effect manifested by the incidence of forestomach lesions, the NOAEL for both sexes of mice was 19 mg/kg. The LOAEL was 38 mg/kg HCCPD. For rats,

Table 5. Incidence[a] of stomach and kidney lesions in mice from Abdo et al. (1984)

	Males						Females					
Dose (mg/kg) Lesion	0	19	38	75	150	300	0	19	38	75	150	300
Stomach Lesions	0/10	0/10	2/10	8/10	9/10	10/10	0/10	0/10	2/9	9/10	10/10	9/9
Toxic Nephrosis	0/10	0/10	0/10	10/10	0/10	0/10	0/10	0/10	0/10	9/10	10/10	7/10

[a] Compared to total number of animals examined.

the NOAEL was 10 mg/kg based on the incidence of forestomach lesions in female rats. The LOAEL for rats was 19 mg/kg.

4.2.2.2. Industrial Biotest Laboratories. 1975b. 90-Day subacute oral toxicity study with C-56 in albino rats. Unpublished report to Hooker Chemical Corporation. Doc # 878212102. NTIS/OTS84003A.

In this study, 0, 30, 100, and 300 ppm HCCPD of unknown purity was fed to 15 weanling male and 15 female Charles River rats per group. The diet was prepared by pre-blending the required amount of HCCPD with the chow in a high-speed blender. Fresh diets were prepared on a weekly basis. No precautions to prevent degradation of the test compound during diet preparation or throughout the study were reported. During the 90-day study, animal weights, food consumption, and clinical signs were recorded. Blood chemistry, hematology, and urinalyses were analyzed at 45 and 84 days. Animals were sacrificed after 90 days, at which time gross examinations, organ weight comparisons, and microscopic examinations were performed.

The authors reported no statistically significant differences between exposed and control populations that were related to HCCPD exposure. On day 45 total leukocyte counts in males and females at 300 ppm were statistically lower than controls (rats at the lower doses were not tested). On day 84, however, male rats at 30 and 100 ppm, and female rats at 100 ppm had statistically higher total leukocyte counts than controls, while total leukocyte counts in both sexes at 300 ppm were not different from controls. Thus, the response did not follow a consistent

dose-response pattern and may be unassociated with HCCPD exposure. Statistical differences in hemoglobin concentration followed that same pattern of dose and duration as those for total leukocyte count. The authors indicated that even though some of the hematologic changes in treated animals were statistically different from controls, the values were still within the limits of normal variation. All other measured parameters, including food consumption, body weight gain, organ weights, hematology, clinical blood chemistry, and urinalyses revealed no exposure-related differences between control and exposed populations.

The results of this study identify a NOAEL of 300 ppm HCCPD in food for male and female rats. Multiplying the total food consumed by the amount of HCCPD in food (i.e., 300 mg HCCPD/kg) and dividing by the number of days on the study (i.e., 90 days) yielded an average daily consumption of 6.9 mg HCCPD/day for males and 5.0 mg HCCPD/day for females. Dividing the average daily consumption of HCCPD by the average weight of the animals yielded NOAEL doses of 21.4 mg/kg/day for males and 25 mg/kg/day for females. However, since the HCCPD was not tested for degradation throughout the study and the HCCPD/food mixture was prepared only on a weekly basis, the stability of the test compound is in question. The absence of observable effects in this study could be a direct result of the degradation of the compound from exposure to light after diet preparation.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

There are no animal studies available on developmental or reproductive effects of HCCPD after inhalation exposure. The following studies suggest a lack of teratogenic effects following oral exposure, although degradation of the highly photoreactive HCCPD may have occurred in some studies.

4.3.1. Murray, F.J., Schwetz, B.A., Balmer, M.F., and Staples, R.E. 1980. Teratogenic potential of hexachlorocyclopentadiene in mice and rabbits. Toxicol Appl Pharmacol 53:497-500.

HCCPD was tested for teratogenicity by administration to an unspecified number of pregnant CF-1 mice and New Zealand white rabbits via oral gavage in cotton seed oil on gestation days 6–15 for mice or 6–18 for rabbits. The test doses were 0, 5, 25, or 75 mg/kg. Mice were sacrificed at gestation day 18 and rabbits were sacrificed at gestation day 29. Gas chromatography indicated the HCCPD preparation was stable for at least 7 days.

No significant effects were seen for number of implantations, fetus viability, resorptions, or mean fetal body measurements. Maternal toxicity in the form of severe diarrhea and subsequent death in an unspecified number of rabbits was seen at 75 mg/kg. A dose-related increase in the proportion of rabbit fetuses with 13 ribs was seen and was statistically significant in the 75 mg/kg group. Due to the authors' statement that 12 or 13 ribs in this species is normal, this increase is not considered to be a significant effect. No other dose-related effects on incidence of fetal malformations in mice or rabbits were seen. The authors concluded that HCCPD was not teratogenic in mice or rabbits at the doses given.

4.3.2. Chernoff, N, Kavlock R.J. 1983. A teratology test system which utilizes postnatal growth and viability in the mouse. Environ Sci Res 27:417-427.

The teratogenicity of HCCPD was tested in mice using a simple screening procedure based on the assumption that prenatal effects would be manifested as changes in two easily measured postnatal parameters (pup viability and growth). This assay was performed with a number of chemicals and found to predict the results of standard, more labor-intensive teratogenicity tests with sufficient accuracy. Twenty-five pregnant CD-1 mice were gavaged with 45 mg/kg HCCPD on gestation days 8–12, the period of major organogenesis. Gestation was allowed to continue until delivery at day 19.

No significant differences in maternal weight change, pup survivorship, or average pup weight were seen between treated animals and untreated controls. The authors conclusion that HCCPD was not a teratogen under the conditions of this assay agrees with the results of the standard mouse assay in Murray et al. (1980).

4.3.3. Goldenthal, E.I., Jessup, D.C., Rodwell, D.E. 1978. Teratology study in rats. Unpublished report by International Research and Development Corporation for Velsicol Chemical Corporation. Report No. 163-573. Doc #40-8249076, NTIS/OTS0512884.

The Velsicol Chemical Corporation performed teratogenicity studies with HCCPD in CD rats (Goldenthal et al., 1978). Groups of 25 pregnant rats were administered doses of 0, 3, 10, or 30 mg/kg HCCPD via corn oil gavage on gestation days 6–15 and were sacrificed on day 20. No significant maternal effects were seen, and no significant fetal effects were seen as measured by mean number of implantations, corpora lutea, live fetuses, post-implantation losses, mean fetal body weights, fetal sex ratios, or incidence of soft-tissue or skeletal malformations. No details were provided on possible precautions taken to prevent compound degradation during the experiment.

4.4. OTHER STUDIES

4.4.1. Contact Dermatitis

Several studies have evaluated the dermal toxicity of HCCPD in rabbits and guinea pigs. A preliminary study involved painting 300 mg/kg HCCPD on the skin (location unspecified) and sacrificing the animal after 24 hours (HEW, 1978). Gross pathology revealed subcutaneous edema from the inguinal region to the mediastinal area. Rib impressions on the parietal surface were apparent from expanded lungs. Histopathology of the lungs revealed atelectasis with thickened alveolar walls containing moderate numbers of macrophages and neutrophils. Histopathology of the skin revealed that the squamous epithelium was one cell thick. No hyperkeratosis or mitotic activity or necrosis of epithelial cells was apparent. Collagen bundles were disrupted by moderate edema and focal pockets of neutrophils were seen in the dermis. Both the dermis and the adipose tissue layer were edematous.

A second preliminary study using doses of 0, 300, 600, and 1,200 mg/kg painted on the skin (location unreported) of one guinea pig/dose resulted in adverse effects similar to those observed in acute oral studies in which rats had been administered up to 300 mg/kg HCCPD in corn oil via gavage. These effects included sneezing, erythema of the eyelids and ears, rhinitis,

cyanosis of the lips and feet, retraction of the head, and labored breathing. In addition, the guinea pigs had black, crusty lesions at the point of HCCPD application (HEW, 1978). The animal dosed with 1,200 mg/kg died 6 hours after treatment.

Treon et al. (1955) applied various solutions of 93.3% HCCPD in Ultrasene to the intact skin of a monkey and two guinea pigs to determine the concentration that produced dermal irritation. When applied to the back of the monkey, 0.05 ml of the 20% solution discolored the skin immediately. After five days, the skin was slightly swollen and after 12 days the skin was scaly. The 10% solution applied to the abdomen produced no signs of irritation. Thus, the threshold concentration for producing dermal irritation in monkeys is between 10% and 20% HCCPD. When applied to the back of a guinea pig, solutions of HCCPD up to 1% produced no effects. On another guinea pig, the lowest concentration tested which produced an effect was 40%. The skin became hard, encrusted and necrotic. Thus, the threshold concentration for irritating the skin of guinea pigs is between 1% and 40% HPCCD.

A 28-day dermal toxicity test was performed using 0.1 and 0.5% HCCPD (w/v) dissolved in denatured ethyl alcohol (Industrial Biotest Laboratories, 1975a). The solutions were applied five days/week for four weeks to the shaved skin of 5 female and 5 male rabbits. These doses were equivalent to 1 mg/kg and 5 mg/kg, respectively. The skin of two males and two females in each group was abraded. After the first application, a slight red erythema was noticeable. After the seventh application, focal necrosis, escharosis, hemorrhaged fissures, and pustules with odorous exudate were reported in both dose groups. Slight-to-moderate (1 mg/kg) or moderate-to-severe (5 mg/kg) desquamation was observed after 20 applications. No deaths occurred, and although a few of the animals in the high dose group lost weight at 14 days (corresponding to the severity of the skin reactions), the animals regained the weight as the lesions healed and formed scabs and scars. No treatment-related effects were reported on hematology, blood chemistry, urinalyses, or gross or microscopic pathology tests.

4.4.2. Genotoxicity

A battery of *in vitro* and *in vivo* genotoxicity studies performed by the National Toxicology Program yielded generally negative results for HCCPD (NTP, 1994). Absence of mutagenicity observed in Ames reversion assays using *Salmonella typhimurium (S. typhimurium)* strains TA98, TA100, TA1535, and TA1537, with or without S9 fraction confirmed earlier results by Industrial Biotest Labs (1977) and Shell (1983). NTP (1994) also obtained negative results for micronucleated erythrocyte frequency in the B6C3F1 mice exposed to HCCPD for 13 weeks by inhalation, and for induction of sex-linked recessive lethal mutations in male *Drosophila melanogaster*. The negative results in *Drosophila melanogaster* essentially duplicated earlier analyses (Zimmering et al., 1985; Mason et al., 1992). When administered to male flies at 10–40 mg/kg in feeding solutions, or at 900–2,000 mg/kg by injection, HCCPD did not increase the number of lethal mutations in male *Drosophila* when compared to controls. However, cytogenetic effects manifested as sister chromatid exchanges and chromosomal aberrations were observed in Chinese hamster ovary cells exposed to HCCPD, with and without S9 (NTP, 1994).

Shell (1983) used a preincubation protocol suitable for volatile chemicals to incubate five strains of *S. typhimurium* (TA1535, TA1537 TA1538, TA98, and TA100) with HCCPD at concentrations up to 10 µg/ml (37 µM) in the absence of S9 fractions, or 500 µg/ml (1.8 mM) in the presence of S9 fractions. There was no evidence of mutagenesis. Similar results were obtained when *S. typhimurium* strain TA100 was incubated for 30, 60, or 120 minutes in the presence of HCCPD as a volatilate at 500–2,500 µg/ml (183 mM–917 mM; Industrial Biotest Laboratories, 1977). As the exposure duration was increased over 120 minutes, cell survival decreased at each concentration tested, indicating that HCCPD is cytotoxic in this concentration range. HCCPD did not induce chromosome damage in metaphase stage rat liver (RL4) cells after a 24-hour incubation at 0.2 µg/ml (0.8 µM), the highest non-toxic concentration tested (Shell, 1983). HCCPD did not induce a significant increase in morphological transformation in BALB/3T3 cells (at concentrations up to 0.000156 µl technical grade HCCPD/ml incubation medium, or 1.6×10^{-5}%) and did not induce forward mutations in mouse lymphoma cells at non-

cytotoxic concentrations (up to 0.00125 µL technical grade HCCPD/ml incubation medium, or 1.3×10^{-4}%) (Litton, 1978). HCCPD at subtoxic concentrations also did not induce DNA repair when incubated with rat hepatocytes *in vitro* (Brat, 1983).

4.4.3. Acute Toxicity

The acute toxicity of HCCPD via inhalation and oral exposure is well established. Treon et al. (1955) performed the only published study for these exposure routes in several different animal species. The lethal dose of a 93.3% pure solution of HCCPD (5% V/V in peanut oil) administered via gavage to female rabbits ranged between 420 and 620 mg/kg. The authors also administered the same solution of HCCPD at doses of 180 to 2,100 mg/kg to groups of ten six month-old rats per dose. The numbers of deaths and adverse effects were recorded for 10 days. The LD_{50} for male rats was 505 mg/kg. Rats and rabbits that died exhibited diffuse degenerative changes in the brain, heart, liver and adrenal glands, degeneration of the liver and kidney tubules, and pulmonary hyperemia and edema. An earlier study using Spartan albino rats administered HPCCD in corn oil at 10 ml/kg body weight (Wazeter and Geil, 1972). The results yielded a LD_{50} of 630 mg/kg for males and 530 mg/kg for females, with a combined LD_{50} for both sexes of 584 mg/kg. The purity of the HCCPD was not reported for this study.

Industrial Biotest Laboratories (1975c) investigated the acute inhalation toxicity for HCCPD (unreported purity) using groups of five male and five female Charles River rats exposed to 2.5 to 21 ppm (28.2–237 mg/m^3)[4] HCCPD for four hours. The LC_{50} was estimated as 38.4 mg/m^3. Necropsies performed on animals that died revealed acute pneumonia with the lungs showing varying degrees of hepatization. Surviving rats were emaciated and often the lungs did not collapse when the thorax was opened. This phenomenon suggests a chronic proliferative inflammatory response in the lungs.

Wazeter and Geil (1972) also studied acute inhalation toxicity of HCCPD (purity unreported) using two sets of 10 male Carworth CFE rats. The rats inhaled either 2 or 200 mg/L

[4]Calculated using conversion of 1 ppm = 11.3 mg/m^3.

(2000 or 200,000 mg/m^3, respectively) HCCPD for 4 hours. All died within 48 hours of exposure. Clinical signs included eye squint, dyspnea, cyanosis, salivation, lacrimation, ocular and nasal porphyrin discharge, and erythema followed by blanching and hypoactivity. Necropsy revealed congestion of the lungs in all rats at the low dose, while rats at the high dose had gray coloring of the skin, and severe hemorrhage of the lung and hydrothorax.

Treon et al. (1955) performed acute inhalation toxicity studies on guinea pigs, rats, mice, and rabbits. The concentrations ranged from 1.7 mg/m^3 (89.5% HCCPD) to 804 mg/m^3. The duration of exposure was increased in some experiments with lower doses (e.g., 3.6 mg/m^3 was administered five times with each exposure lasting seven hours). Clinical signs and fatalities were recorded. LC_{50}s were not estimated. A concentration of 143 mg/m^3 for three hours resulted in fatalities among rabbits, rats, and mice, but not among guinea pigs. The authors noted that rabbits appeared to be the most susceptible species, with mice, rats and guinea pigs exhibiting decreasing susceptibility, in that order. Exposure to concentrations as low as 3.6 mg/m^3 irritated the eyelids and increased respiratory rate after two or three days (species not indicated). Prolonged intermittent exposure (150 exposures of seven hours each) to 1.7 mg/m^3 HCCPD, the lowest concentration administered, resulted in slight degenerative changes in the livers and kidneys of all species observed. Mice exhibited pulmonary edema and bronchitis, and some of the guinea pigs and rats developed pneumonia (incidence not specified). The rabbits did not appear to manifest an inflammatory response at 1.7 mg/m^3.

Ulrich and Hagan (1978) administered HCCPD (unknown purity) at 8 different concentrations from 0.28 to 5.8 ppm (3.2 to 66 mg/m^3)[5] to groups of 10 male and 10 female Sprague-Dawley rats. The experiment consisted of inhalation exposure to HCCPD for 4 hours, followed by a 14-day observation period. The 4-hour LC_{50} was 18 mg/m^3 for male rats and 41.3 mg/m^3 for females, which indicated that males are more sensitive to the compound. The LC_{50} for females was similar to the 38.4 mg/m^3 LC_{50} calculated by Industrial Biotest Labs (1975c) using both sexes. Ulrich and Hagan (1978) observed some degree of sedation in all rats exposed

[5]Calculated using conversion of 1 ppm = 11.3 mg/m^3

to 16 mg/m^3 or greater, and dyspnea in all animals at 40 mg/m^3 or greater. Tearing, salivation, and ataxia were observed in most animals exposed to 66 mg/m^3. All animals in the 3.2 mg/m^3 group gained weight normally over the 14-day observation period while animals in all other exposure groups (16–66 mg/m^3) lost weight. Necropsies indicated that animals exposed to 16 mg/m^3 or greater had red focal or diffuse consolidation of the lungs progressing to severe generalized hemorrhage and hepatization that was dose-dependent. Some animals in the 66 mg/m^3 group also had rhinorrhea and mottling of the liver. The authors noted that despite the biphasic mortality curve (indicating potentially two toxic responses), only pulmonary abnormalities were found.

These studies indicate that HCCPD vapors are very toxic and cause respiratory effects during repeated exposures to low concentrations such as 1.7 mg/m^3. Treon et al. (1955) indicated that the acute inhalation toxicity of HCCPD was greater than that of phosgene.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN)—ORAL AND INHALATION

There are no epidemiologic data concerning the chronic health effects of HCCPD alone in humans. Mortality studies from several plants at which HCCPD was used cannot distinguish between the effects from exposure to HCCPD and effects from exposure to the other chlorinated compounds present. Nevertheless, mortality studies reported no increases in death from any causes, including cancer, for employees exposed to HCCPD and other chlorinated chemicals compared to matched populations from the U.S. (Brown et al., 1980; Buncher et al., 1980; Shindell, 1980; Shindell, 1981; Wang and MacMahon, 1979).

An occupational study (Boogaard et al., 1993) of the chronic effects of HCCPD followed more sensitive health measures than the mortality studies but has the same problem, i.e., the effects of HCCPD cannot be distinguished from those of other chemicals to which the subjects were exposed. Male chemical plant operators exposed to HCCPD (0.11 mg/m^3), allyl chloride (3 mg/m^3), 1,3-dichloropropene (<5 mg/m^3), and epichlorohydrin (< 4 mg/m^3) for an average of 8.2 years did not show any differences in liver and kidney function tests as compared to controls.

The data indicate that chronic exposure to this mixture of chlorinated solvents did not cause significant liver or kidney damage under these occupational exposure conditions.

An acute occupational exposure to HCCPD at concentrations that may have been as high as 211 mg/m^3 produced eye irritation, headache, persistent fatigue, chest discomfort, skin irritation, and cough that persisted for up to 6 weeks following exposure (Kominsky et al., 1980). Liver function studies on workers detected slight increases in serum glutamate-oxalacetate transaminase, alkaline phosphatase, total bilirubin, and lactate dehydrogenase. These changes indicate that acute exposure to high concentrations of HCCPD may result in liver damage. However, the relationship of HCCPD to hepatotoxicity is confounded by inadequate pre-exposure monitoring, the presence of OCCP, and the lack of definitive exposure data.

Three developmental toxicity studies showed that oral HCCPD did not induce adverse developmental effects in mice, rats, or rabbits, even at doses which induced severe maternal toxicity such as diarrhea and subsequent death in rabbits (Murray et al., 1980; Chernoff and Kavlock, 1983; Goldenthal et al., 1975). Oral doses as high as 75 mg HCCPD/kg were tested.

The metabolic pathways of HCCPD are not well known. Pharmacokinetic studies in mice, rats and rabbits indicate that absorption, distribution and excretion of HCCPD depends on exposure route. Orally administered HCCPD is poorly absorbed (Mehendale, 1977; Yu and Atallah, 1981; Lawrence and Dorough, 1981, 1982). Although the relative concentration varies with route, the kidneys, liver and lungs are the predominant sites for HCCPD distribution. Oral HCCPD concentrates mainly in the kidneys, followed by the liver, and then the lung (Lawrence and Dorough, 1981; 1982). Distribution studies involving both rats (Lawrence and Dorough, 1981; 1982) and mice (Dorough and Ranieri, 1984) indicate that inhaled HCCPD deposits primarily in the trachea, followed by the lungs, and the kidneys. IV HCCPD deposits in the kidneys, followed by the lungs, and then the liver. The exposure route also influences the excretion of HCCPD. Inhaled HCCPD is excreted primarily in the urine, whereas oral HCCPD is excreted mainly via the feces. The larger proportion of excretion via feces after oral administration is due, at least partly, to the larger proportion of biliary excretion. Approximately equal proportions of an IV dose end up in urine and feces. Metabolism of radiolabeled HCCPD

in rodents is rapid, with the majority of the radiolabel excreted within 24 hours of administration (Yu and Atallah, 1981; Lawrence and Dorough, 1981, 1982; Dorough and Ranieri, 1984). Attempts to characterize the polar metabolites from tissue homogenates or urine or fecal samples have been unsuccessful (Mehendale, 1977; Yu and Atallah, 1981; Shell, 1984).

4.5.1. Inhalation Studies

There are several subchronic inhalation toxicity studies available and there is one study of chronic duration. While no adverse effects were noted in monkeys or rats exposed to up to 2.2 mg/m^3 HCCPD in a subchronic regimen, rats exhibited minor changes in hematologic parameters, which were not dose-related, at exposures as low as 0.11 mg/m^3 after 12 weeks of exposure (Rand et al., 1982a). In another subchronic study, Clark et al. (1982) identified the lungs as a target organ for HCCPD toxicity. Four of 20 rats exposed to 5.5 mg/m^3 HCCPD died from bronchopneumonia. That exposure also produced epithelial hyperplasia, edema, sloughing of bronchiolar epithelium and epithelial ulceration and necrosis. Decreases in body weight were noted at 1.1 mg/m^3. Changes in hematologic parameters with no consistent dose or duration relationship were also noted. A later subchronic study using rats and mice (NTP, 1994) reported none of the hematologic changes noted in the earlier studies, but confirmed the respiratory tract pathology. Necrotic and suppurative inflammation of the lung occurred in male rats exposed to 4.5 mg/m^3 HCCPD. Higher exposures, 11 and 22 mg/m^3, produced more severe lesions such as extensive coagulation necrosis in the epithelium of the respiratory tract, inflammatory signs and 100% mortality. Mortality (3/20) was observed in mice exposed to doses as low as 0.45 mg/m^3 in the absence of respiratory tract histopathology (NTP, 1994). The 2-year NTP (1994) study found no respiratory tract pathology in rats exposed to up to 2.3 mg/m^3 HCCPD or in male or female mice exposed to up to 0.56 mg/m^3. At 2.3 mg/m^3, mice exhibited suppurative inflammation of the nose. A dose-related increase in the incidence of suppurative ovarian inflammation was seen in female mice, but it was not considered to be the critical effect since it was attributed to a Klebsiella infection. Neither rats nor mice showed any evidence of exposure-related carcinogenicity.

4.5.2. Oral Studies

Only subchronic studies are available for the oral route of exposure. HCCPD administered in feed for 90 days produced no effects in rats at doses of up to 21-25 mg/kg/day (Industrial Biotest Labs, 1975). The actual delivered dose in this study is questionable, however, because the stability of HCCPD in the weekly prepared diet was not verified. HCCPD administered via gavage for 13 weeks was responsible for rat mortality at doses as low as 75 mg/kg and mouse mortality at 300 mg/kg (Abdo et al., 1984). Forestomach lesions were observed at 19 mg/kg in female rats and at 38 mg/kg in male rats and both sexes of mice (Abdo et al., 1984). Toxic nephrosis was seen at 38 mg/kg in both sexes of rats and at 75 mg/kg in female mice. Although they did not develop toxic nephrosis at any dose, male mice developed acute tubular necrosis at 300 mg/kg. The other major toxic effect in this study was significantly reduced body weight beginning at 38 mg/kg in rats and 150 mg/kg in mice. No adverse effects were noted at 19 mg/kg in mice or at 10 mg/kg in rats.

4.5.3. Mode of Action

HCCPD is a relatively reactive chemical as evidenced by its portal of entry effects. The biological reactivity of HCCPD may be a result of its reactivity in Diels-Alder reactions in which it combines with an alkene (a dienophile) in a cycloaddition reaction (ATSDR, 1999). Potential biological reactants with HCCPD include quinones, sterols, 2-alkenoic acids, unsaturated fatty acids and unsaturated fatty acid derivatives. HCCPD can also undergo addition and substitution reactions or be oxidized by the mixed function oxidase system.

4.6. Weight of Evidence Evaluation and Cancer Classification—Synthesis of Human, Animal, and Other Supporting Evidence; Conclusions About Human Carcinogenicity and Mode of Action

Mortality studies suggest that occupational exposure to HCCPD (and the other chlorinated compounds to which workers were exposed) does not produce an increase in deaths from cancer (Brown et al., 1980; Buncher et al., 1980; Shindell, 1980, 1981; Wang and

MacMahon, 1979). One 2-year inhalation carcinogenesis study reported no increase in the incidence of tumors in rats or mice at doses up to 2.2 mg/m^3 (NTP, 1994). This study involved a 5-day-per-week dosing regimen, which is acceptable as relevant to human exposure. Further, the study was well designed and involved two rodent species and an appropriate number of subjects at each dose. The study did report a statistically significant incidence of benign adenoma in the pituitary in male rats exposed to a concentration of 2.2 mg/m^3 HCCPD, but since the incidence was only slightly greater than historical controls, it was not considered to be biologically significant. A significant increase in the incidence of squamous metaplasia of the larynx in female rats was also noted, but this effect was not dose-dependent. NTP (1994) concluded that there was no evidence of carcinogenic activity.

A number of mutagenicity assays with HCCPD have been negative. Exposure of five strains of *S. typhimurium* to concentrations up to 1.83 µM, in the presence or absence of microsomal fractions, produced cytotoxicity, but not mutagenicity (EPA, 1994). HCCPD also produced negative results in mouse micronucleus assays (NTP, 1994) and showed no evidence of transformation of BALB/3T3 cells or forward mutations in mouse lymphoma cells (Litton, 1978). HCCPD did not induce DNA repair when incubated with rat hepatocytes (Brat, 1983) or induce lethal mutations in the offspring of male *Drosophila* (Zimmering et al., 1985; Mason et al., 1992). The only positive result for mutagenicity was a significant increase in sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells.

The apparent inability of HCCPD to cause genotoxic effects, and the lack of evidence for both human and animal carcinogenicity, justify the conclusion that HCCPD is not likely to present a human cancer risk. According to the existing Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), the evaluation of the overall weight-of-evidence for carcinogenicity to humans indicates that HCCPD is most appropriately characterized as Group E —Evidence of Noncarcinogenicity to Humans. This characterization is based on the lack of evidence for carcinogenicity in adequate animal tests in two different species. Human data are inadequate because there are too few pertinent studies. Although the available occupational mortality studies were limited by the low number of cases and confounded by exposures to other

chemicals, no increased deaths from cancer were observed. In accordance with U.S. EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996), HCCPD is not likely to be a human carcinogen. This characterization is based on no evidence of cancer in rodents, lack of mutagenicity and the lack of increased deaths from cancer in the limited human studies available.

4.7. Susceptible Populations

4.7.1. Possible Childhood Susceptibility

There are no human studies that indicate the relative sensitivity of children and adults to the toxic effects of HCCPD. There are no animal inhalation studies for developmental effects, but oral studies which administered HCCPD during organogenesis showed no significant fetal effects (Chernoff and Kavlock, 1983; Goldenthal et al., 1978) even at doses which cause severe maternal effects (Murray et al., 1980). Based on these results, it is unlikely that HCCPD causes teratogenic effects in humans, but its effects on children are unknown.

4.7.2. Possible Sex Differences

Epidemiology studies have not provided adequate information on sex differences in susceptibility to HCCPD toxicity. The mortality studies (Buncher et al., 1980; Wang and MacMahon, 1979; Shindell and Associates, 1980, 1981; Brown et al., 1980) and single occupational cohort (Boogaard et al., 1993) were predominantly limited to men and did not report significant health effects. Subchronic inhalation studies in cynomolgous monkeys reported no sex differences. Several subchronic studies in rodents, however, suggested that female rodents are more sensitive to sublethal effects while males are more sensitive to the lethal effects. Abdo et al. (1984) found more male rodents than female rodents died at the higher doses during a subchronic gavage study, but female rats were more sensitive to forestomach lesions

than male rats, and female mice were more sensitive to toxic nephrosis than male mice. A subchronic inhalation study generally reported that more male mice than females died at doses producing mortality (NTP, 1994). For both rats and mice, males were more sensitive than females to respiratory tract inflammation (NTP, 1994). In the chronic inhalation study, however, there were no clear differences in the sensitivity of male and female rodents. There are no mechanistic data available to support or refute male-female differences in sensitivity in animals and, thus, no way to predict those susceptibilities in humans.

5. DOSE-RESPONSE ASSESSMENTS

5.1. Oral Reference Dose

5.1.1 Choice of Principal Study and Critical Effect with Rationale and Justification

No chronic oral studies for HCCPD were identified. There were two subchronic oral studies in rodents. The dietary study in rats by Industrial Biotest Labs (1975) did not provide information on the stability of weekly prepared HCCPD/food mixture, so the actual dose delivered in this study is questionable. No effects were noted at the highest doses tested: 21.4 mg/kg/day in male rats or at 25 mg/kg/day in female rats. The gavage study by Abdo et al. (1984) didn't report stability of the gavage preparation, but Abdo et al. (1984) is favored over the Industrial Biotest Labs (1975) study because toxic effects were observed. Abdo et al. (1984) had previously been used for deriving the RfD for HCCPD.

Ten F344 rats per sex were administered 0, 10, 19, 38, 75, or 150 mg HCCPD/kg in corn oil by gavage 5 days per week for 13 weeks. Ten B6C3F1 mice per sex were administered 0, 19, 38, 75, 150 or 300 mg HCCPD/kg on the same schedule. Mortality, significant decreases in body weight, and forestomach lesions were observed in all rodents at the higher doses. Toxic nephrosis was also reported in male and female rats and in female mice. The toxic nephrosis was characterized by proximal tubular dilation, cytomegaly, karyomegaly, and anisokaryosis with nuclear and cytoplasmic vacuolization and occurred at doses higher than those producing forestomach lesions. Since forestomach pathology was the most sensitive treatment-related adverse effect, it was identified as the critical effect. The forestomach lesions were characterized

in rats by a varying degree of inflammation associated with hyperplasia in the surface epithelium with the formation of vesicles or bullae, and ulceration and erosion of the mucosa. Lesions in mice mainly consisted of inflammation and proliferation, with ulceration restricted to the highest dose in both sexes. Rats were more sensitive than mice. Forestomach lesions were observed in female rats beginning at 19 mg/kg and in both sexes of mice beginning at 38 mg/kg. The NOAEL for this lesion in female rats was identified as 10 mg/kg and the LOAEL was 19 mg/kg (see Table 5-1).

5.1.2 Methods of Analysis—Benchmark Dose Analysis

The incidence of treated animals with stomach lesions is a quantitative measure of toxicity that allows benchmark dose analysis. Only data from female rats were used because this sex was more sensitive to HCCPD toxicity based on the presence of a response in females at 19 mg/kg, which did not produce a response in males (Abdo et al., 1984). The dose-response data and the conversion to continuous dosing are shown in Table 6. Since Abdo et al. (1984) provided gavage administration 5 days per week, the doses were adjusted to daily doses by multiplying by 5 days/week and dividing by 7 days/week. Thus, the duration-adjusted NOAEL and LOAEL are 7 and 14 mg/kg/day, respectively.

Table 6. Incidence of forestomach lesions in female F344 rats

Administered Dose (mg/kg/day)	Duration-Adjusted Dose (mg/kg/day)[1]	Incidence of Forestomach Lesions
0	0	0/10
10	7	0/10
19	14	2/10
38	27	5/10
75	54	9/10
150	107	9/10

[1] Conversion to adjust for exposure duration (5 days to 7 days),

e.g., 150 mg/kg/day × 5/7 = 107 mg/kg/day

Benchmark dose (BMD) analysis was chosen for dose-response analysis because it uses the entire dose-response curve to identify the point of departure, it does not depend upon dose-spacing, and it is sensitive to the number of animals used in the study. The data available met the suggested criteria (U.S. EPA, 1995) of at least three dose levels with two doses eliciting a greater than minimum and less than maximum response. Nine statistical models from U.S. EPA's Benchmark Dose Software (v1.2) were applied to the data to identify the model that best fit the dose-response curve (see Appendix B). The models with good statistical fit, as evidenced by goodness-of-fit p-values >0.05, were retained for evaluation of visual fit at the lower doses. The model with the best evaluation was used to estimate the BMD_{10} (dose predicted to cause a 10% increase in the incidence of the effect) and the $BMDL_{10}$ (the 95% lower confidence limit on the BMD_{10}). Visual ranking is important to assess whether the calculated curve fits well in the 10% response range.

Six of the nine statistical models met the statistical requirements for goodness of fit: gamma (p = 0.4333), quantal-linear model (p = 0.5784), Weibull model (p = 0.4312), multistage (p = 0.4055), log-logistic (p = 0.7766), and log-probit (p = 0.7368). The log-logistic and log-probit models were chosen to estimate the BMD_{10} and $BMDL_{10}$ since they clearly had the best visual fit at the control and two lowest doses, which encompassed the 10% response. The BMD_{10} and $BMDL_{10}$ calculated by these models were nearly identical. The BMD_{10}s for the log-logistic and log-probit models were 10.57 and 10.56 mg/kg/day and the $BMDL_{10}$s were 5.6 and 5.98 mg/kg/day. Both models yield a $BMDL_{10}$ of 6 mg/kg/day when rounding to one significant figure (see Appendix B).

5.1.3 RfD Derivation, Including Application of Uncertainty Factors (UFs) and Modifying Factors (MFs)

Uncertainty factors (UFs) are applied to the BMD_{10} and $BMDL_{10}$ to account for uncertainties in extrapolation from rodent bioassay data to human exposure conditions, for unknown variability in human sensitivities, for data deficiencies, and for other factors. Historically, UFs were applied as values of 10 in a multiplicative fashion (Dourson and Stara,

1983). Recent EPA practice, however, also includes use of a partial UF such as $10^{1/2}$ (U.S. EPA, 1994b) under conditions where toxicokinetics and mechanistic information are available and/or data are available on the nature and extent of human variability.

Chronic studies are preferred for RfD development. To account for the uncertainty in using a subchronic study for RfD derivation, the default UF of 10 is usually applied; however, for HCCPD, the ratio of subchronic to chronic NOAEL for the inhalation studies are used to determine the subchronic to chronic UF. This approach is justified by the fact that HCCPD produces local effects by both routes of exposure. The subchronic inhalation study of NTP (1994) observed a NOAEL of 1.7 mg/m^3 for respiratory effects in rats while the chronic study observed a NOAEL of 2.23 mg/m^3. Since comparing the subchronic NOAEL for inhalation exposure in rats to the chronic NOAEL yielded counterintuitive results, i.e., the subchronic NOAEL was less than the chronic NOAEL, the mouse results were examined. The subchronic mouse bioassay (NTP, 1994), yielded a NOAEL of 1.7 mg/m^3 while the NOAEL in the chronic assay was 0.56 mg/m^3 HCCPD. Thus, the subchronic:chronic ratios for NOAELs in mice is 3. Thus, to be conservative, the subchronic to chronic UF for the RfD is 3, rather than 1.

The toxicokinetics of HCCPD are not well understood, and it is not known if the toxicity is due to the parent compound or to metabolites. However, it is known that HCCPD does not bioaccumulate and tissue concentrations and excretion of the compound depend somewhat on the exposure route. Rodent and rabbit studies show that oral HCCPD is absorbed rather poorly and excreted largely in the feces (about 70% of a single dose), but because there is no information on which to base a pharmacokinetic or pharmacodynamic comparison of animals to humans, the default UF of 10 is used for interspecies extrapolation. There are no data documenting the nature and extent of variability in human susceptibilities to HCCPD, so the default UF of 10 is used to protect sensitive human subpopulations. The database for HCCPD includes studies of genotoxicity, developmental toxicity, systemic toxicity, and cancer, but no two-generation reproductive studies are available. An additional UF of 3 is added for this database deficiency. Thus, the total UF is 1000 (3 subchronic to chronic NOAEL, 10 for interspecies variability, 10 for interspecies variability, and 3 for database deficiency).

The BMD_{10} and $BMDL_{10}$ are divided by the total UF of 300 to derive the RfD.

$$BMD_{10} = 10.6 \div 1000 = 0.011 \text{ mg/kg/day}$$

$$BMDL_{10} = 6 \div 1000 = 0.006 \text{ mg/kg/day}$$

Thus, the RfD, as derived from the $BMDL_{10}$, is 0.006 mg/kg/day.

5.2. Inhalation Reference Concentration

5.2.1. Choice of Principal Study and Critical Effect with Rationale and Justification

Only one chronic inhalation study for HCCPD was identified. NTP (1994) exposed rats and mice to 0, 0.11, 0.56, and 2.23 mg/m^3 for 5 days/week for 2 years. Exposure to HCCPD did not affect survival in rats or in male mice. The survival of female mice in the 2.23 mg/m^3 group was marginally lower than controls. Squamous metaplasia of the larynx was noted in female rats at 0.11 and 2.23 mg/m^3 HCCPD, but it was not dose-related. No adverse effects were noted in male rats. Exposure-related effects in mice included suppurative inflammation of the nose in both sexes at 2.23 mg/m^3. Female mice exhibited suppurative inflammation of the ovaries that increased in a dose-dependent fashion. The effect was observed at 0.11 mg/m^3 HCCPD, but began to be statistically significant at 0.56 mg/m^3. The slightly lower survival rate for female mice in the 2.23 mg/m^3 group was attributed to the ovarian inflammation. It was not considered to be the critical effect since it was thought to be due to Klebsiella infection and because several subchronic inhalation studies (NTP, 1994; Clark et al., 1982; Rand et al., 1982a) had identified the respiratory system as the major target of HCCPD toxicity. Thus, the suppurative inflammation of the nose in mice was used as the critical endpoint for calculation of the RfC.

The dose-response data for suppurative inflammation of the nose for male and female mice reported in the NTP (1994) study, and the duration adjustment to continuous exposure are shown in Table 7. The NOAEL for suppurative inflammation of the nose was 0.56 mg/m^3 and the LOAEL was 2.23 mg/m^3. Adjusting from intermittent to continuous exposure results in a duration-adjusted NOAEL of 0.1 mg/m^3 and a LOAEL of 0.4 mg/m^3.

Table 7. Incidence of suppurative inflammation of the nose in mice

Exposure Concentration (mg/m^3)	Duration-Adjusted Exposure (mg/m^3)[1]	Nasal Inflammation Incidence
0	0	4/99
0.1	0.02	0/100
0.56	0.10	4/100
2.23	0.40	76/98

[1] Conversion from intermittent exposure to continuous exposure:

$0.56\ mg/m^3 \times 6/24\ hrs \times 5/7\ days = 0.10\ mg/m^3$.

5.2.2. Methods of Analysis—NOAEL/Benchmark Concentration Analysis

Benchmark concentration (BMC) analysis is preferred for dose-response analysis because it uses the entire dose-response curve to identify the point of departure, it does not depend upon dose-spacing, and it is sensitive to the number of animals used in the study. The available data, however, did not meet the suggested criteria (U.S. EPA, 1995) of at least three dose levels with two doses eliciting a greater than minimum and less than maximum response. Thus, the duration-adjusted NOAEL of 0.10 mg/m^3 is used to derived the RfC.

HCCPD is a Category 1 gas (U.S. EPA, 1994b) since its effects by inhalation target the respiratory tract. The human equivalent concentration (HEC) for HCCPD is derived by multiplying the duration-adjusted NOAEL for rodents by an interspecies dosimetric adjustment factor for gas:respiratory effects in the region of critical effect. Since the critical effect is in the nose, the dosimetric adjustment factor was calculated for the extrathoracic (ET) region.

For HCCPD, the dosimetric adjustment factor is the regional gas dose ratio (RGDR) for HCCPD in the ET region. The RGDR was calculated as the ratio of mouse to human ventilation rate/ET surface area. The ventilation rate (V_E) was calculated for mice using the average body weight of males and females in the NOAEL exposure group (i.e., 41.4 g). The ventilation rate for mice was calculated as 0.049 L/minute using the allometric relationships contained on page 4-27 of U.S. EPA (1994b). The default human ventilation rate is 13.8 L/minute (U.S. EPA,

1994b). The default ET surface areas (SA_{ET}) for the mouse and for the human are shown in Table 4-4 of U.S. EPA (1994b) as 3.0 and 200 cm^2, respectively. The RGDR was calculated as follows:

$$RGDR_{ET} = (V_E / SA_{ET})_{animal} / (V_E / SA_{ET})_{human} = (0.049/3.0) / (13.8 /200) = 0.237$$

The duration-adjusted NOAEL was then multiplied by the $RGDR_{ET}$ to yield the $NOAEL_{HEC}$:

$$NOAEL_{HEC} = NOAEL_{ADJ} \times RGDR_{ET} = 0.1 \text{ mg/m}^3 \times 0.237 = 0.024 \text{ mg/m}^3$$

5.2.3. RfC Derivation Including Application of Uncertainty Factors (UFs) and Modifying Factors (MFs)

Uncertainty factors (UFs) are applied to the $NOAEL_{HEC}$ to account for uncertainties in extrapolation from rodent bioassay data to human exposure conditions, for unknown variability in human sensitivities, for data deficiencies, and for other factors. Historically, UFs were applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). Recent EPA practice, however, also includes use of a partial UF such as $10^{1/2}$ (U.S. EPA, 1994b) under conditions where toxicokinetics and mechanistic information are available and/or data are available on the nature and extent of human variability.

For long-term rodent bioassays, the default uncertainty factors for interspecies extrapolation and within-species variability are each 10. Half of that factor, $10^{1/2}$, or 3, reflects the pharmacokinetic component of uncertainty and half represents the pharmacodynamic component of uncertainty. The calculation of an HEC adjustment to the NOAEL reduces the uncertainty associated with interspecies variation. Therefore, the use of UF = 3, instead of the default UF = 10, is justified for interspecies extrapolation. There are no data documenting the nature and extent of variability in human susceptibility; therefore, the default UF of 10 is used for within-species variation. No data are available on the developmental or reproductive effects of

HCCPD after inhalation exposure. The inhalation toxicity database for HCCPD is, therefore, judged to be limited, and an additional uncertainty of 3 is used in the calculation of the RfC.

A total uncertainty factor of 100 (3 for interspecies variability, 10 for intraspecies variability, and 3 for a limited database) is applied to the $NOAEL_{HEC}$ of 0.024 mg/m^3, yielding an RfC of 0.0002 mg/m^3.

5.3. Cancer Assessment

Human occupational studies and animal studies have failed to demonstrate an association between exposure to HCCPD and cancer. The NTP conducted a 2-year inhalation study with rats and mice, and concluded that HCCPD exhibited no evidence of carcinogenic activity (NTP, 1994). HCCPD is not likely to be a human carcinogen due to the absence of increased deaths from cancer in limited human studies, no evidence of cancer in rodents, and lack of mutagenicity. Therefore, a quantitative dose-response assessment for carcinogenicity has not been conducted for HCCPD.

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD IDENTIFICATION AND DOSE-RESPONSE ASSESSMENTS

6.1. Hazard Identification

HCCPD is a dense oily liquid, pale yellow to amber in color. It has a pungent, unpleasant odor. It is predominately used as an intermediate in the production for many compounds used as dyes, resins, pharmaceuticals, flame retardants, insecticides, and polyester resins. HCCPD is also used to produce ketones, fluorocarbons, acids, esters, and shock-proof plastics.

In animals, HCCPD is absorbed poorly after oral exposures, but is absorbed readily following inhalation exposures. Oral HCCPD is excreted mainly in the feces while inhaled HCCPD is excreted primarily in the urine. Metabolism is poorly characterized. The distribution of the compound and metabolites depends somewhat upon exposure route, but the kidneys, liver and lungs are the major tissues of concentration regardless of route of exposure. HCCPD and metabolites are typically excreted within a few days of dosing and do not accumulate in tissues.

No repeated-exposure human toxicity data exists for HCCPD that do not also involve exposures to other compounds. In animals, the compound adversely affects the histopathology of the tissues along the portal of entry. Inhalation exposure produces inflammation and hyperplasia in the nose, larynx, trachea, and lung of treated rodents exposed for 13 weeks at doses as low as 4.5 mg/m^3 (NTP, 1994). A longer term study using lower doses found only suppurative inflammation of the nose at doses as low as 2.3 mg/m^3. Gavage administration for 13 weeks induced mild to moderate forestomach lesions and toxic nephrosis in rats and mice (Abdo et al., 1984). The lowest dose producing these effects was 19 mg/kg. No significant developmental effects were observed via oral exposure in three studies using mice, rats, or rabbits at doses as high as 75 mg/kg during organogenesis (Goldenthal et al., 1978; Murray et al., 1980; Chernoff and Kavlock, 1983).

The potential carcinogenic effects of HCCPD have been studied in rodents (NTP, 1994). In a 2-year study with rats and mice, no treatment-related neoplastic lesions were observed. Generally, *in vitro* and *in vivo* mutagenicity tests have produced negative results. According to the existing Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), HCCPD is most appropriately characterized as a Group E, Evidence of Noncarcinogenicity to Humans, carcinogen. This characterization is based on inadequate data for cancer in humans and evidence of noncarcinogenicity in animals. In accordance with U.S. EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996), HCCPD is not likely to be a human carcinogen due to the absence of increased deaths from cancer in limited human studies, no evidence of noncarcinogenicity in rodents, and lack of mutagenicity.

6.2. Dose Response

The RfD of 0.006 mg HCCPD/kg/day was derived from a 13-week subchronic bioassay (Abdo et al., 1984), in which rats and mice exhibited forestomach histopathology at the highest three doses tested. Forestomach lesions in female mice were identified as the critical effect. An overall uncertainty factor of 1000 was applied to the $BMDL_{10}$ to account for the subchronic exposure, extrapolation from rat to human, and intrahuman variability.

The overall confidence in the oral RfD is low; however, the confidence in the principal study is medium. Although it was well conducted, an adequate number of doses were examined, and corroborative results in two species were obtained, no data on hematology, clinical chemistry or urine analyses were collected. In addition, there are no supporting subchronic or chronic oral studies with which to compare the effects noted. Teratogenic studies are available for three species, but confidence in the database in low due to the lack of a chronic study and a two-generation reproductive study.

The teratology studies using oral administration of HCCPD during organogenesis reported no occurrence of adverse effects in mice, rats, or rabbits. Although these studies may suggest that HCCPD does not produce developmental effects, no multi-generational reproductive studies have been performed to examine development for stages other than organogenesis.

The daily inhalation exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime (RfC) is 0.0002 mg/m^3. This value was derived from a 2-year inhalation assay by NTP (1994). Dose-related suppurative inflammation of the nose was observed in mice. An overall uncertainty factor of 100 was used to account for the limited database, extrapolation from mouse to human, and for intrahuman variability.

The overall confidence in the RfC assessment is medium. The confidence in the principal study is high because it was well designed and well conducted and followed standard guidelines for inhalation toxicity studies of chronic duration. The overall confidence in the database is medium. Although there are two subchronic studies which verify that the respiratory tract is the major target organ, the database lacks reproductive/developmental studies in rodents following inhalation exposure to HCCPD. Oral teratogenicity studies in three species, however, indicate that HCCPD is not teratogenic at doses (i.e., 75 mg/kg) higher than those which cause portal of entry irritation (i.e., 19 mg/kg). This suggests that the possible teratogenic effects of inhaled HCCPD may be less sensitive than respiratory tract effects.

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APPENDIX A

External Peer Review—Summary of Comments and Disposition

APPENDIX B

Benchmark Dose Calculations for the RfD

The RfD is based on forestomach lesions in the female rat, as reported in Abdo et al. (1984). The dose-response data and the conversion to continuous dosing are shown below in Table B-1.

Table B-1. Incidence of forestomach lesions in female F344 rats

Administered Dose (mg/kg/day)	Continuous Dose (mg/kg/day)	Incidence of Forestomach Lesions
0	0	0/10
10	7	0/10
19	14	2/10
38	27	5/10
75	54	9/10
150	107	9/10

[1] Conversion to adjust for exposure duration (5 days to 7 days),
e.g., 150 mg/kg/day x 5/7 = 107 mg/kg/day

NOAEL = 7 mg/kg/day

LOAEL = 14 mg/kg/day

The $BMDL_{10}$ (95% lowest confidence limit of the dose predicted to cause a 10% increase in the incidence of the effect) was estimated using U.S. EPA's Benchmark Dose Software (Version. 1.2). The results of applying nine statistical models for dichotomous data from BMDS to the data for mild to moderate forestomach lesions are shown in Table B-2. Models with statistical goodness-of-fit p-value > 0.05 were evaluated for visual fit in the low dose region, which approximates 10% response. The gamma, quantal-linear, Weibull, multistage, log-logistic, and log-probit models had adequate statistical goodness-of-fit. The log-logistic and log-probit models clearly had the best visual fit at the control and two lowest doses, which encompassed the 10% response, and the values for BMD and BMDL were nearly identical.

Table B-2. Benchmark Dose Results for Forestomach Lesions

Model	Chi-Square Goodness-of-Fit p-Value	Visual Rank	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)
Gamma	0.4333	2	8.97	3.57
Logistic	0.0	NE	24.8	24.3
Log-logistic	0.7766	1	10.56	5.6
Multistage	0.4055	4	5.41	3.13
Probit	1	NE	51.87	11.29
Log-probit	0.7368	1	10.57	5.98
Quantal-linear	0.5784	4	4.37	3.07
Quantal-quadratic	0.0000	NE	14.44	11.82
Weibull	0.4312	3	7.39	3.35

NE-Not evaluated because statistical goodness of fit p-value was < 0.05.

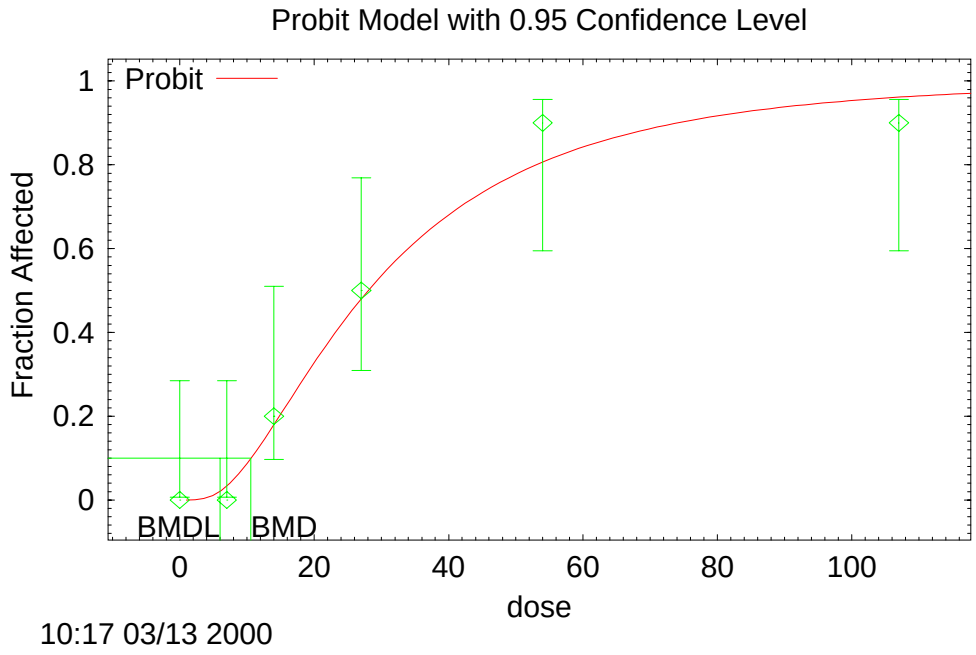
The BMD_{10} of 10.6 mg/kg/day and the $BMDL_{10}$ of 6 mg/kg/day were divided by the UF of 1000 to derive the RfD.

BMD_{10}= 10.6 ÷ 1000 = 0.011 mg/kg/day

$BMDL_{10}$= 6 ÷ 1000 = 0.006 mg/kg/day

Graphical results from the BMD models that were visually ranked follow.

Log Probit–Visual rank=1



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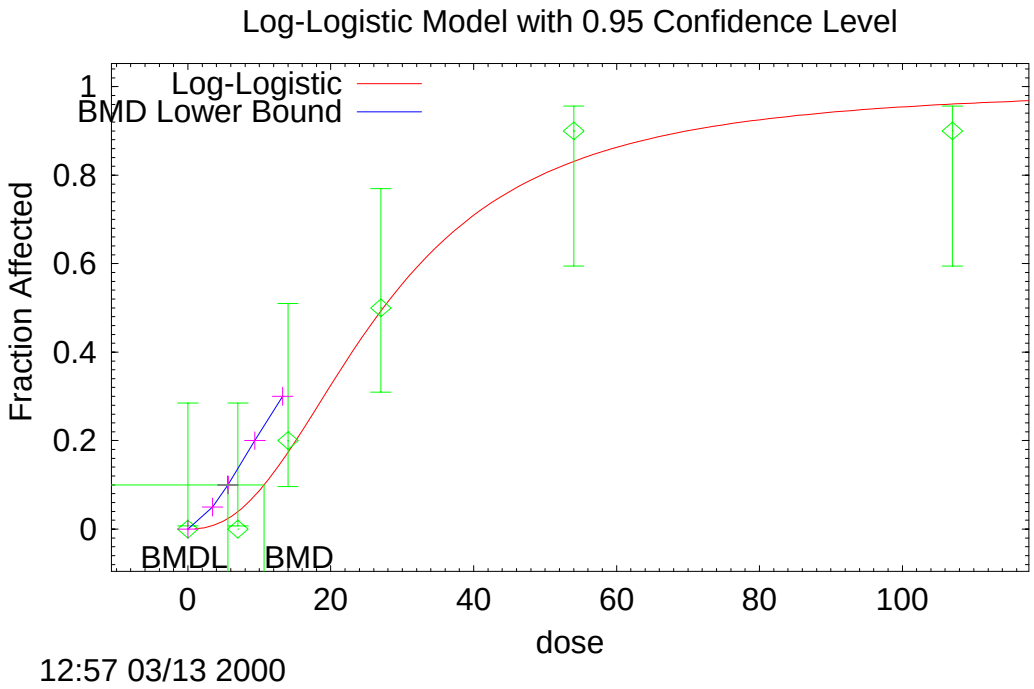

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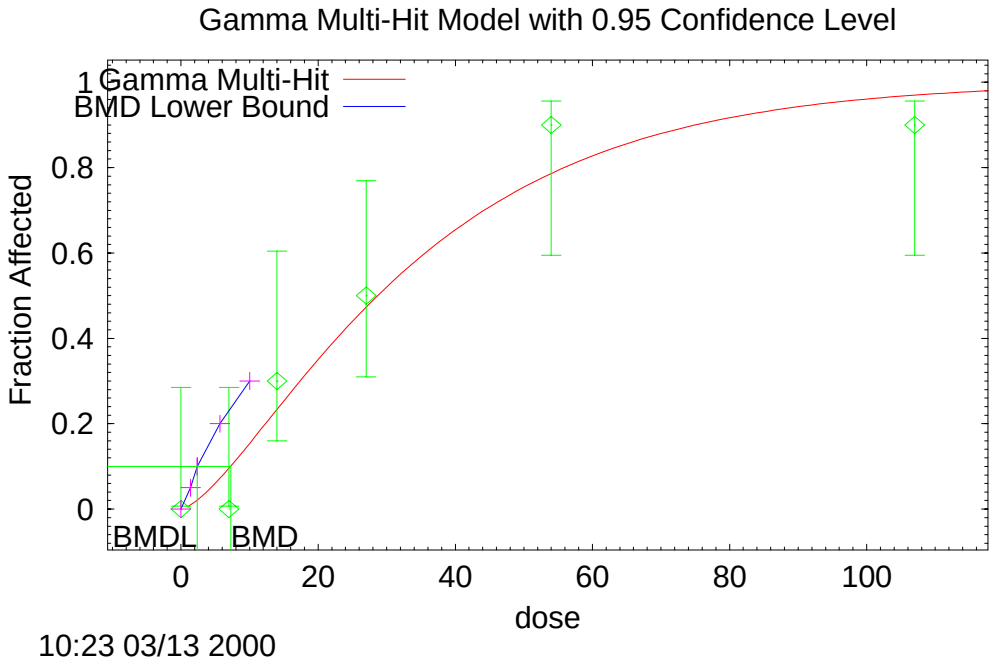

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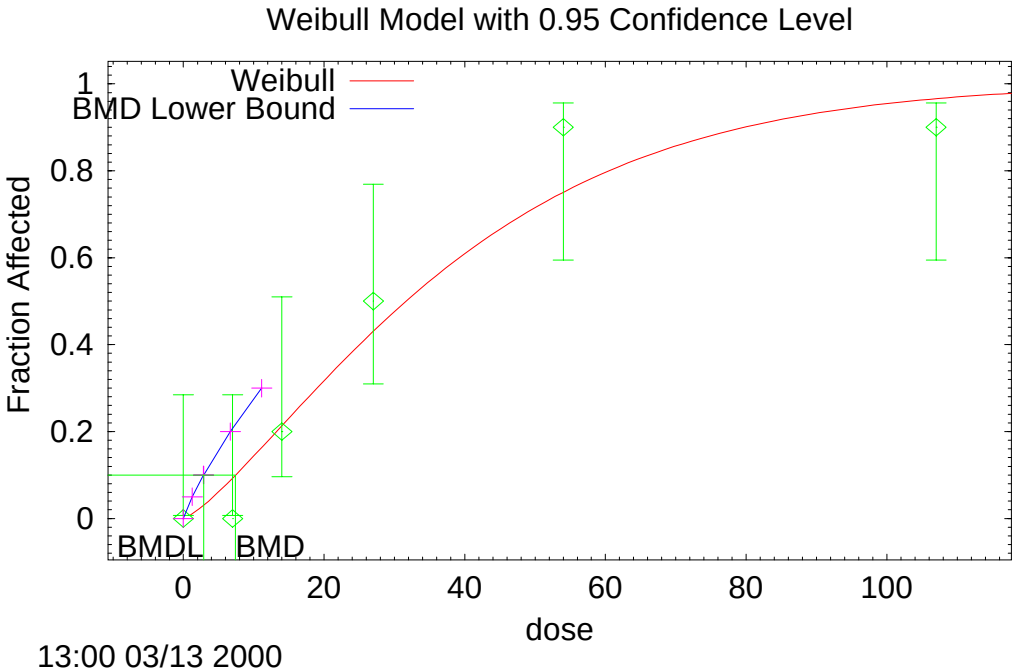

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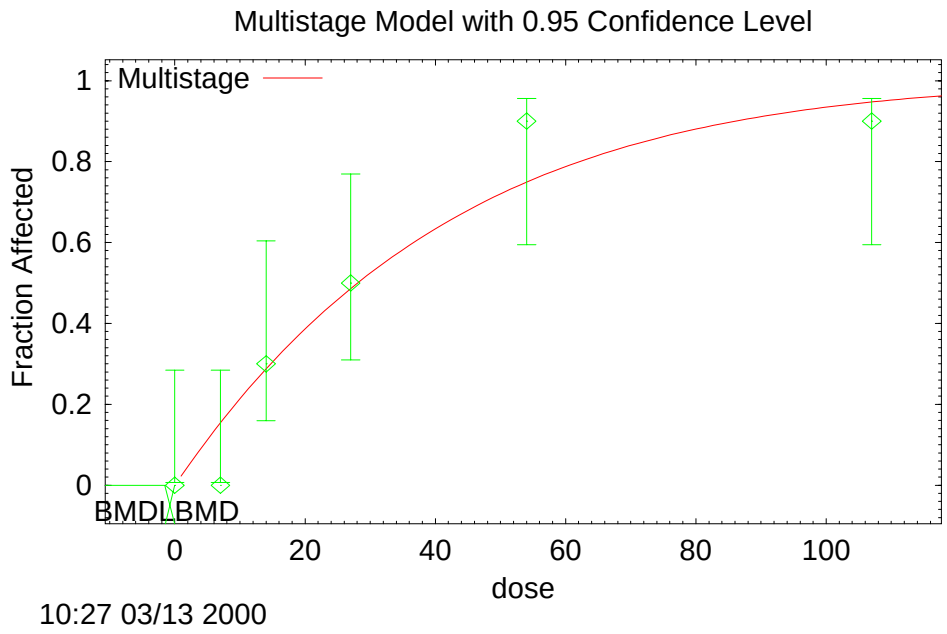

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